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INFECTION AND DISSEMINATION EXPERIMENTS WITH DEGENERATION DISEASES OF POTATOES. OBSERVATIONS IN 1923[1]

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INTRODUCTION

In a former publication[2] evidence was presented indicating that degeneration of the potato (*Solanum tuberosum* L.) is brought about by at least seven diseases, for which the etiology remains to be demonstrated. It was established that although these diseases are quite similar regarding transmission, they differ sufficiently in transmission and symptoms to be considered distinct, even in the same variety and in the same environment. Furthermore, it was shown that combinations of these diseases occur, usually causing more severe injury than a single disease. Insect transmissibility was proved for six diseases and for several combinations.

These results suggested that further investigations might disclose additional similar maladies; that some unsolved apparent combinations might be reduced to still simpler forms; that further knowledge of the reactions of different varieties to those single diseases and their combinations was desirable; that additional information on insect carriers, including different genera or species of aphids and other insects, deserved further attention; and that additional data on alternate hosts should be obtained. In order to secure further evidence on these and similar problems relating more or less directly to control, the investigations presented in this paper were conducted.

DESCRIPTION OF SYMPTOMS

The symptoms of the different diseases to be considered will now be indicated. Four types of mosaic, mild, leaf-rolling, rugose, and crinkle mosaic, are recognized. Mild mosaic (pl. 6, A, 3; B, 4 and pl. 7, A, 2) is characterized chiefly by distinct mottling, wrinkling, some ruffling, and slight dwarfing in the foliage, and by reduction in the yield rate of tubers without modification of their shape or color. Infection with juice inoculations is more difficult with mild mosaic than with rugose mosaic or streak. Leaf-rolling mosaic (pl. 6, A, 2; B, 2, and pl. 9, C) differs from mild mosaic in its diffuse mottling and in a slight rolling of the middle and upper leaves; it resembles mild mosaic in the difficulty of infection by juice inoculations. Tuber symptoms resemble those of mild mosaic. Leaf-rolling mosaic is distinguished from leaf-roll (pl. 10, A) by the absence of distinct rolling, dwarfing, or rigidity, and of chlorosis. Rugose mosaic (pl. 6, A, 1; B, 1; pl. 7, A, 1; and pl. 8, C and D) is characterized by somewhat diffuse mottling, distinct dwarfing, rugosity, and a shorter life period (pl. 7, A, 1) and in current-season or late previous season infections (pl. 8, C) by brittleness, spotting, and streaking, resulting in abnormally premature death. Tuber reduction is more marked than in mild mosaic. Infection by means of leaf mutilation is easier with rugose mosaic

[1] Received for publication May 20, 1924; issued June, 1925. This paper is based upon investigations conducted as a cooperative project between the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology of the Maine Agricultural Experiment Station. Unless otherwise indicated, the work was performed in northeastern Maine. The order of arrangement of the authors' names is not intended to indicate that one cooperating institution contributed more than the other to the results.

[2] SCHULTZ, E. S., and FOLSOM, D. TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF IRISH POTATOES. Jour. Agr. Research 25: 43–118, illus., 1923.

than with mild mosaic. What seems to be a fourth type of mosaic, resembles mild mosaic but is characterized by a much greater degree of crinkling, that is, of wrinkling, waviness of the margin, and ruffling. This will be called "crinkle mosaic" (Pl. 6, B, 3). It is not identical or even similar to the "crinkle" of other systems of nomenclature. The latter (crinkle) seems to be nearly identical with rugose mosaic, but in descriptions based upon symptom aggregates in plants of the Green Mountain variety, "crinkle" can not replace the well-known term mosaic, and furthermore rugose mosaic is characterized by less crinkling than some other types of mosaic, at least in the variety and environment most familiar to the writers.

The distinguishing characteristics of leaf-roll (Pl. 10, A) include distinct rolling and rigidity, especially of the lower leaves, dwarfing, chlorosis, and some burning. Tubers, usually quite sessile, are reduced in size. They may, in certain varieties and conditions, show net necrosis resulting in spindlingness of the sprouts, especially at the stem end. Spindlingness of the sprouts also may be a symptom without net necrosis (pls. 5 and 7).

Spindle tuber produces spindling stems and upright, somewhat darker green and slightly rugose and dwarfed foliage. Abnormally spindling, spindle-shaped, and usually dwarfed tubers with more or less conspicuous and apparently numerous eyes, are characteristic of this disease. Infection with spindle tuber is obtained with about the same difficulty as with mild or leaf-rolling mosaic.

The characteristic current season symptoms of streak (pls. 2, 3 and 4, A) include streaking, spotting, brittleness, leaf dropping, and premature death of the foliage. The streaking and spotting are more pronounced on the lower leaf surface than the upper, if they appear on the latter. With the exception of faint chlorotic spots marking the initial stages of streaking and spotting, no mottling develops. Second generation reactions of streak appear as severe dwarfing, curling, wrinkling, brittleness, streaking, leaf dropping, and premature death, often preventing tuber development. Brown discolored areas frequently appear near the eyes on infected tubers. Streaks and spots have been observed on the white corollas of inoculated plants of the Green Mountain variety (pl. 3).

Mottled curly dwarf (pls. 4, C, and 5, E) symptoms include distinct dwarfing, spindlingness, curling, wrinkling, slight rolling, brittleness, burning, and somewhat premature death. The tubers show the same symptoms as in spindle tuber, with more dwarfing. This symptom aggregate appears to be a combination of leaf-rolling mosaic and spindle tuber. Unmottled curly dwarf (pl. 1, A, B, and C) is different from mottled curly dwarf in the absence of mottling and in the tubers being more gnarled and cracked. This seems to be a single disease. The tuber cracking may appear, without the spindlingness, as a symptom resulting from current-season inoculation (pl. 1, A to D).

SECOND GENERATION REACTIONS TO INOCULATIONS MADE IN 1922

LEAF MUTILATION INOCULATIONS MADE WITHIN THE GREEN MOUNTAIN VARIETY IN THE FIELD

In the open field in 1922, leaf mutilation inoculations with different types of mosaic and with leaf-roll, spindle tuber, unmottled curly dwarf, and streak, were performed on plants of the Green Mountain, Irish Cobbler, Bliss Triumph, and Rural New Yorker varieties. Either the second or third hill, or both of these hills, in each four-hill tuber unit were inoculated, leaving two or three uninoculated controls in each tuber unit. Single inoculations were made in each series, with the exception of series 38, 58, and 73, in which the second hill in each tuber unit received two applications, and with the exception of series 71, which received three

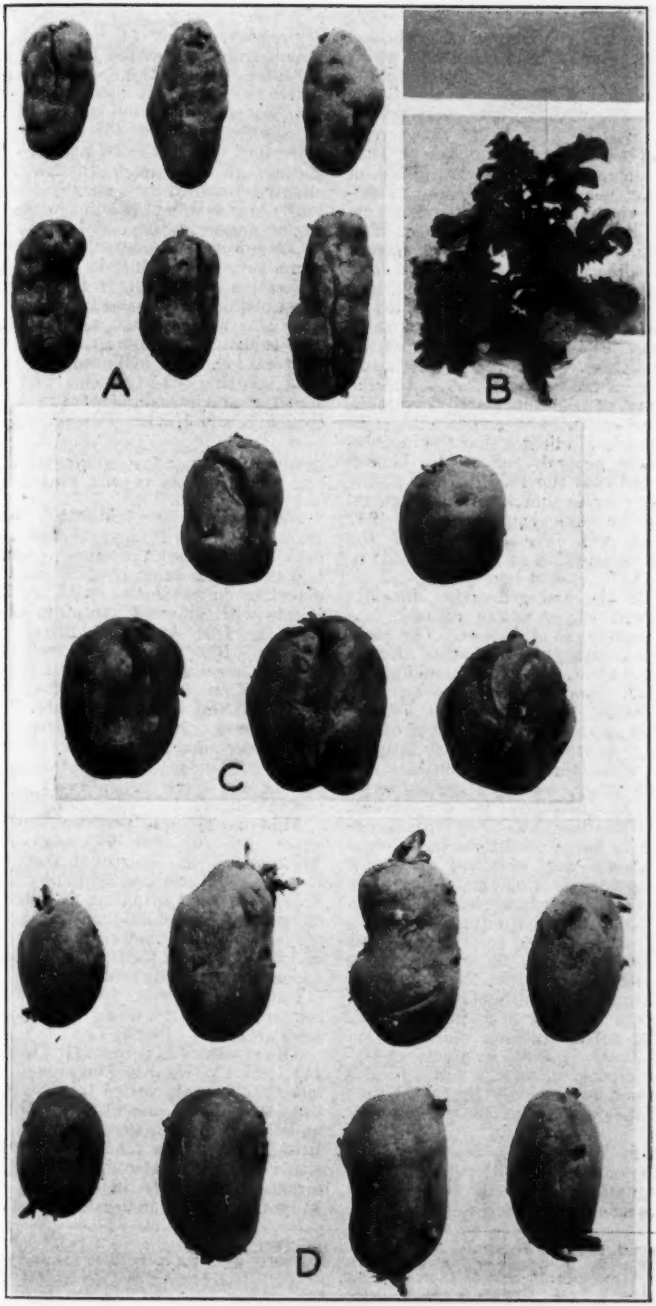
EXPLANATORY LEGEND FOR PLATE 1

A.—Tubers produced by unmottled curly-dwarf Green Mountain plants in a lot serving as the source of inoculum for inoculation series 22 of Table I. For the effects of the inoculation see Plate 1, B, C, and D. Photographed at planting time in 1923

B.—Unmottled curly dwarf plant grown from one of the cracked tubers shown in Plate 1, C. Photographed in August, 1923

C.—Tubers produced by Green Mountain plants inoculated in series 22 of Table I with unmottled curly dwarf, showing cracks as a current-season symptom. Photographed at planting time in 1923. For the complete tuber symptoms see Plate 1, A, for the controls see Plate 1, D, and for the vine symptoms see Plate 1, B

D.—Tubers produced by sister hills of the plants inoculated with unmottled curly dwarf in series 22 of Table I. Four tubers are split and the entire surface of each tuber is shown. One recently formed transverse bruise crack is evident, but is different from the longitudinal healed growth cracks symptomatic of diseased plants (pl. 1, C). Photographed at planting time in 1923



(For explanatory legend see p. 494)

treatments. A number of series showed current season symptoms in 1922 which are noted in a previous paper.[3] Only inoculations performed in the Green Mountain variety are included in Table I.

The disease recorded in the controls was contracted because the plants of the first generation grew close to diseased vines with transmitting insects of at least one species present. Mild mosaic, rugose mosaic, and spindle tuber spread more to the controls than did the other diseases. Inoculations with mild mosaic are not included in Table I, since most of the controls in these series became infected with the same disease. However, the high percentage of mild mosaic observed in the progeny of the inoculated plants in comparison with the percentage in other series, indicates that the inoculation was generally effective. It will be noted that the 1923 progeny of the lots serving as sources of inoculum retained the same symptoms as the 1922 parents, with the exception of the apparent addition of rugose mosaic in series 38 from field infection.

As in the first generation, inoculations with rugose mosaic resulted in a high percentage of disease. The characteristic symptoms of this disease resembled those in the inoculated plants in 1922. Rugose mosaic occurred in combination in series 10, 38, 79, 83, and 127 apparently as a result of inoculation. Another combination inoculation, with leaf-rolling mosaic and spindle tuber, was obtained in series 136. As indicated in a previous paper[3] modifications of symptoms occurred in these combinations. Negative results were obtained with juice from plants having glabrous, more or less crisp and fleshy leaves suggesting mutation,[4] except for the spindle tuber originally in combination. As indicated, in series 95, spindle tuber was associated with the mutation, becoming obvious when the source of inoculum was dug in 1922. In series 22 the tuber cracking of unmottled curly-dwarf appeared as a current-season symptom without the tuber spindlingness, followed by the usual vine and tuber symptoms in the progeny (pl. 1, A, B, C, and D).

As in 1922, streak occurred alone and in combination with rugose mosaic. Perpetuation of streak through the tubers soon ceases because of the lack of progeny. However, a strain of streak from one source has been perpetuated without any apparent combination or change, through four succeeding seasons by means of leaf mutilation inoculation of a new lot each season. Therefore streak is to be regarded as a distinct disease, even though it is closely resembled by a streaking which may occur as a first-season symptom of rugose mosaic, as previously recorded.[5] It is noteworthy that streak alone causes more severe dwarfing in the second generation than results from most combinations of degeneration diseases (pl. 2). The writers have not yet been able to distinguish more than one kind of streak, this one including leaf dropping, spotting of the corollas, and both spotting and streaking of the leaves (pls. 2, 3, and 4, A).

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN THE FIELD

Intervarietal leaf mutilation inoculations were performed in the open field in 1922 with the same procedure and under the same conditions as are described in connection with Table I. Plants with different symptom aggregates in Irish Cobblers, Rural New Yorkers, Bliss Triumphs, and three different seedling lots, served as sources of inoculum for the inoculation of healthy Green Mountain plants. The current-season reactions to these treatments are described in a previous paper.[6] The second generation reactions are given in Table II of the present paper.

Mild mosaic was produced only in series 42, 46, and 54, with Bliss Triumph as the source of inoculum. In series 54 the Bliss Triumph foliage showed marked crinkling in addition to the apparent mild mottling. As indicated, this symptom complex was manifested also in the inoculated Green Mountain plants as crinkle mosaic.

The results with leaf-roll, as in former leaf-mutilation inoculations, were negative. (Series 71.)

The reactions in series 111, 113, 117, 119, and 121 regarding mosaic in the inoculated plants varied from those in the source of inoculum; although mottling could not be detected in the Rural New Yorker plants, it appeared either as the leaf-rolling or rugose mosaic type in the inoculated Green Mountain plants, indicating a varietal

[3] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVII.
[4] FOLSOM, D. MUTATIONS OF THE POTATO. TWO SOMEWHAT UNSTABLE LEAF-FORM SPORTS OF THE IRISH POTATO. Jour. Heredity 14: 45–48, illus., 1923.
[5] SCHULTZ E. S., and FOLSOM, D. Op. cit. p. 79.
[6] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVII.

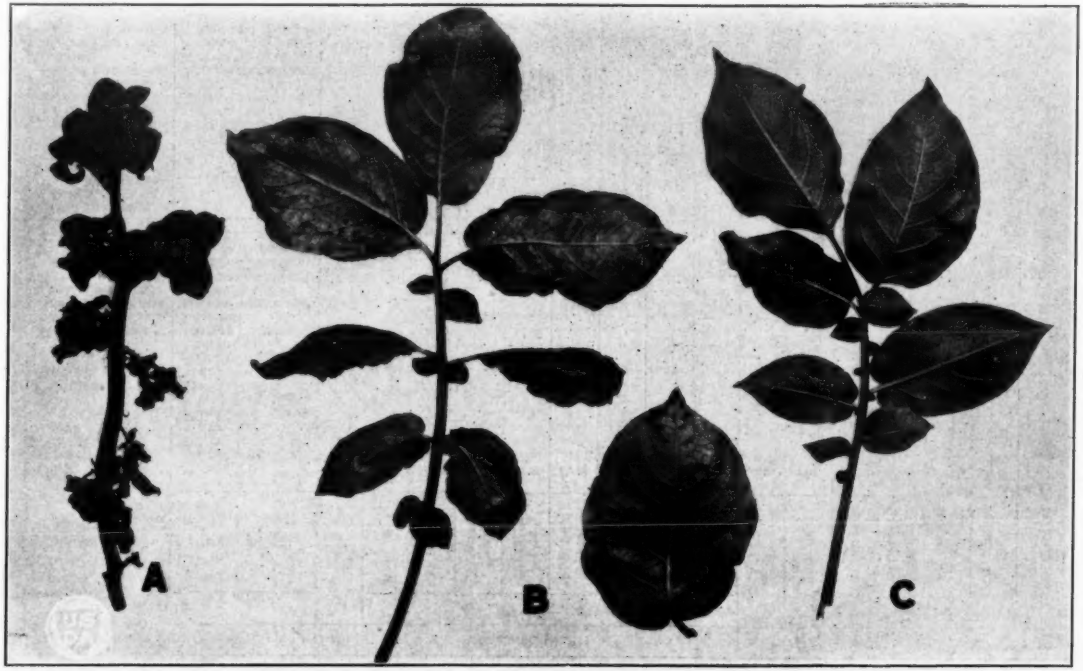
TABLE I.—*Leaf mutilation inoculations within the Green Mountain variety in the open field in 1922 and the progeny in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants									Controls								
	Variety	Symptoms		1922 hills	Progeny, 1923								1922 hills	Progeny, 1923							
		1922	Progeny, 1923		Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Leaf-roll		Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Healthy
				No.	*No.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*No.*	*No.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
10	Green Mountain.	Rugose mosaic and streak.	No progeny	5	35	.....	.....	100	.....	.....	71	.....	5	20	50	.....	.....	.....	.....	5	45
14	....do.....	Streak	....do.....	5	24	8	.....	4	.....	.....	87	.....	5	[a] 20	15	.....	30	.....	.....	.....	50
22	....do.....	Unmottled curly dwarf.	Unmottled curly dwarf.	5	36	.....	.....	.....	.....	52	.....	.....	5	[b] 20	40	10	25	.....	.....	.....	15
26	....do.....	Spindle tuber	Spindle tuber	10	69	.....	.....	.....	76	.....	.....	.....	30	60	.....	.....	.....	36	.....	.....	64
38	....do.....	Medium mosaic	Mild, rugose, and leaf-rolling mosaic.	10	65	32	.....	41	.....	.....	.....	.....	10	20	15	.....	20	.....	.....	.....	65
58	....do.....	Mosaic medium+	Rugose mosaic	10	59	.....	.....	100	.....	.....	.....	.....	5	20	25	.....	15	.....	5	.....	55
73	....do.....	Leaf-roll	Leaf-roll	5	39	.....	.....	.....	.....	.....	.....	.....	5	20	.....	.....	.....	.....	.....	.....	100
79	....do.....	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	5	53	.....	.....	100	100	.....	.....	.....	5	20	.....	.....	.....	.....	.....	.....	100
83	....do.....	Rugose and leaf-rolling mosaic.	Rugose and leaf-rolling mosaic.	5	35	.....	100	100	.....	.....	.....	.....	5	20	.....	.....	.....	.....	.....	.....	100
87	....do.....	Rugose mosaic	Rugose mosaic	5	39	.....	.....	100	.....	.....	.....	.....	5	20	.....	.....	.....	.....	.....	.....	100
91	....do.....	Water control	.....	5	32	.....	.....	.....	.....	.....	.....	.....	10	36	.....	.....	.....	.....	.....	.....	100
95	....do.....	Thick-leaf mutation and spindle tuber.	.....	5	27	.....	.....	.....	63	.....	.....	.....	5	16	.....	.....	.....	12	.....	.....	88
96	....do.....	Thick-leaf mutation.	.....	5	29	.....	.....	.....	.....	.....	.....	.....	5	20	.....	.....	.....	.....	.....	.....	100
97	....do.....	Unmottled curly dwarf.	Unmottled curly dwarf.	5	25	.....	.....	.....	.....	48	.....	.....	5	20	.....	5	.....	.....	.....	.....	95
101	....do.....	Leaf-rolling mosaic.	Leaf-rolling mosaic.	5	30	.....	33	.....	.....	.....	.....	.....	5	20	.....	.....	.....	.....	.....	.....	100
122	....do.....	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	5	28	.....	.....	[c]	85	.....	.....	.....	5	10	.....	.....	.....	.....	.....	.....	100
127	....do.....	Rugose mosaic and streak.	Rugose mosaic and streak.	5	41	.....	.....	68	.....	.....	68	.....	5	10	.....	.....	.....	.....	.....	.....	100
136	....do.....	Leaf-rolling mosaic and spindle tuber.	Leaf-rolling mosaic and spindle tuber.	10	61	.....	100	.....	65	.....	.....	.....	10	20	.....	.....	.....	20	.....	.....	80

[a] 5 per cent small plants.

[b] 20 per cent small plants. Some units with more than one type of mosaic

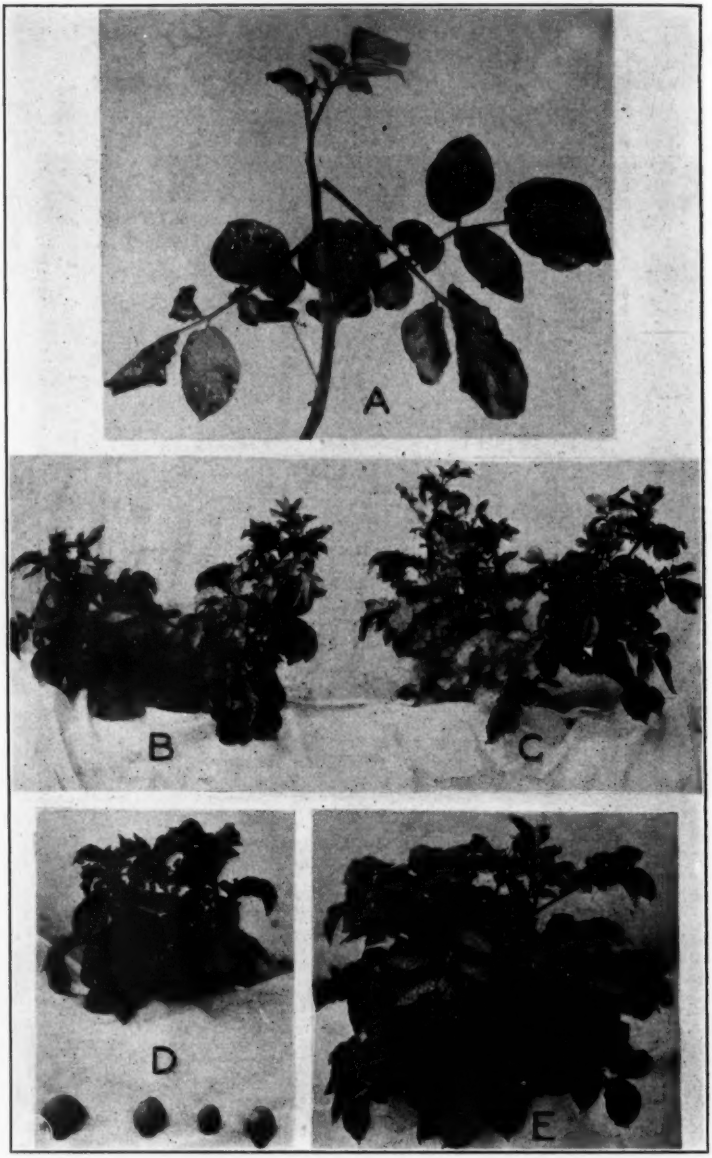
[c] Some.



Second-year streak (A), current-season symptoms of leaf mutilation inoculation with streak in cages (B), and a healthy control leaf (C) from a sister hill to that represented by "B." Green Mountains, photographed on Sept. 6, 1923

Second-year streak (A), current-season symptoms of leaf mutilation inoculation with streak in cages (B), and a healthy control leaf (C) from a sister hill to that represented by "B." Green Mountains, photographed on Sept. 6, 1923

Above from left to right, withered flower, open flowers, bud, and leaflet, showing spotting and streaking as the result of current-season leaf-mutilation inoculation with streak. Below, flowers and leaflet from an uninoculated hill of the same tuber unit as the above. Green Mountains, photographed on Aug. 4, 1922



A.—Current-season symptoms of leaf mutilation inoculation with streak in the open field. Green Mountain, photographed on Aug. 4, 1923

B and C.—Unmottled ruffled dwarf Rural New Yorker hill (B) representing the source of inoculum of series 113 of Table II, and Green Mountain hill (C) representing the progeny of the inoculated plants of this series. The Green Mountains showed a leaf-rolling-mosaic spindle-tuber combination commonly designated as curly-dwarf (distinct from unmottled curly dwarf). Photographed on Aug. 27, 1923. Compare with Plate 1, B, Plate 4, E, and Plate 5, E

D.—Unmottled curly dwarf Rural New Yorker hill representing the source of inoculum of series 111 of Table II. Photographed on Aug. 27, 1923. See Plate 5, D for the Green Mountains of this series

E.—Irish Cobbler hill representing the source of inoculum of series 139 of Table II. Photographed on Aug. 11, 1923. See Plate 5, E for the Green Mountains of this series

TABLE II.—*Intervarietal leaf mutilation inoculations of Green Mountains in the open field in 1922 and their progeny in 1923.*

Inoculation series No.	Source of inoculum: Variety	Source of inoculum: Symptoms 1922	Source of inoculum: Symptoms Progeny, 1923	Inoculated plants: 1922 hills	Inoculated plants: Progeny, 1923: Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Leaf-roll	Controls: 1922 hills	Controls: Progeny, 1923: Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Healthy
				No.	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	No.	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
30	Irish Cobbler	Spindle tuber	Spindle tuber	10	72				68				30	57				12			88
42	Bliss Triumph	Medium mosaic	Mild mosaic	10	67	50							5	20	20						80
46	do	Slight + mosaic	do	10	62	59							5	20	45						55
54	do	Medium + mosaic	Mild and rugose (?) mosaic.	5	37	[a] 87							5	20	60						40
71	Irish Cobbler	Leaf-roll	Leaf-roll	10	59								5	19							100
111	Rural New Yorker	Curly dwarf and spindle tuber.	Curly dwarf and spindle tuber.	5	31			100		100			5	10							100
113	do	Ruffle dwarf and spindle tuber.	Ruffle dwarf and spindle tuber.	5	33		72		97				5	10	40			10			60
115	do	Dwarf; spindle tuber.	Dwarf; spindle tuber.	5	36				69				5	10							100
117	do	Ruffle dwarf in upper leaves.	Ruffle dwarf in upper leaves.	5	36		100						5	10		10					90
119	do	Mottled curly dwarf and spindle tuber.	Rugose curly dwarf.	5	31		100	100					5	10	50						50
120	do	Healthy	Healthy.	5	[b] 37								5	10							100
121	do	Ruffle dwarf in upper leaves.	Ruffle dwarf	5	30		100						5	10							100
123	Seedling 39374	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	5	41			100	100				5	10				10			90
130	Seedling 41451	Streak and slight rugosity on young leaves.		5	32			100					5	10							100
133	Seedling 41491	Streaking		5	37			100					5	10							100
139	Irish Cobbler	Mild mosaic (?) and spindle tuber.	Leaf-rolling masaic and spindle tuber.	5	22		100	(c)	100				5	10							100
141	do	Leaf-rolling mosaic, rugose mosaic, and spindle tuber.	Leaf-rolling mosaic, rugose mosaic, and spindle tuber.	5	31		100	(c)	100				5	10							100
142	do	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	6	31			100	100				5	10							100

[a] 54 per cent with crinkling.

[b] Healthy.

[c] Some rugose mosaic.

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modification of symptoms. These observations confirm similar phenomena recorded previously.[8] It appears that distinct ruffling of the foliage in the Rurals, even in the absence of mottling, denotes mosaic infection mainly of the leaf-rolling type (pl. 4, B and C). Curly dwarf in Rurals often indicates the presence of rugose mosaic. Curly dwarf in series 111 appeared on Green Mountains as a combination of rugose mosaic and unmottled curly dwarf. (Pls. 4, D, and 5, D.)

Streaking and slight rugosity in the seedling lot in series 130 and 133 apparently are symptoms of rugose mosaic; current-season symptoms in 1922[9] also corroborate these second generation reactions. Series 139, 141, and 142 disclose double and triple combinations of leaf-rolling and rugose mosaic and spindle tuber, with mottling often less conspicuous in Irish Cobblers than in Green Mountains (pls. 4, E and 5, E). Such combinations may become common in originally healthy Cobbler stock grown for several seasons where these various diseases are present, without mosaic being disclosed by mottling.

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN INSECT CAGES

Leaf mutilation inoculations with degeneration disease combinations, between the Green Mountain and other varieties, were performed in insect cages at the same time and in the same plot as those described in Tables I and II. A major series of inoculated plants consisted of 12 plants grown from 3 tubers, each tuber having been quartered. The 4 plants from each tuber were grown respectively in 4 cages and each cage contained 3 plants, or a minor series. Thus the plants in each cage, or minor series, represented all 3 tubers of the major series, and one uninoculated minor series served as a control to the others. At the time of the first inoculation the average height of the plants was about 7 cm. One application was given to each series with the exception of series A–III and B–III which received three inoculations at weekly intervals. The results of these inoculations for 1922 and 1923 are recorded in Table III. Here each major series is denoted by a letter and each minor series or cage by the letter with a Roman numeral.

Repeated inoculations with mild mosaic in series A–III produced a higher percentage of diseased plants than the single inoculation in series E–III between the same varieties, while the same is true of spindle tuber but with a smaller difference. The symptoms in those two series appeared the same regardless of the difference in the number of inoculations. In 1923, spindle tuber appeared in every variety including the seedling. The percentage of infection with this malady was higher than with any other disease represented in these inoculations, even in the series originally healthy as indicated by the controls. No mottling resulted from inoculations with mild mosaic on Irish Cobblers in series B–III, which is in marked contrast to the high percentage of infection obtained with three applications of the same inoculum on Green Mountains in series A–III. Also, with a single inoculation, the slight amount of infection in Green Mountains, Bliss Triumphs, and seedling 39374 was absent in Irish Cobblers.

"Mosaic dwarf" inoculations produced rugose mosaic and spindle tuber in Green Mountains and in Bliss Triumphs but not in Irish Cobblers (considering the controls). In the seedling, one hill showed streak in 1922, followed in 1923 by either no growth or extreme dwarfing. These facts indicate that "mosaic dwarf" may sometimes consist of a combination of rugose mosaic, spindle tuber, and streak. The inoculum from the seedling plants produced spindle tuber in Green Moun-

[8] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 65, 76, 79.
[9] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVII.

EXPLANATORY LEGEND FOR PLATE 5

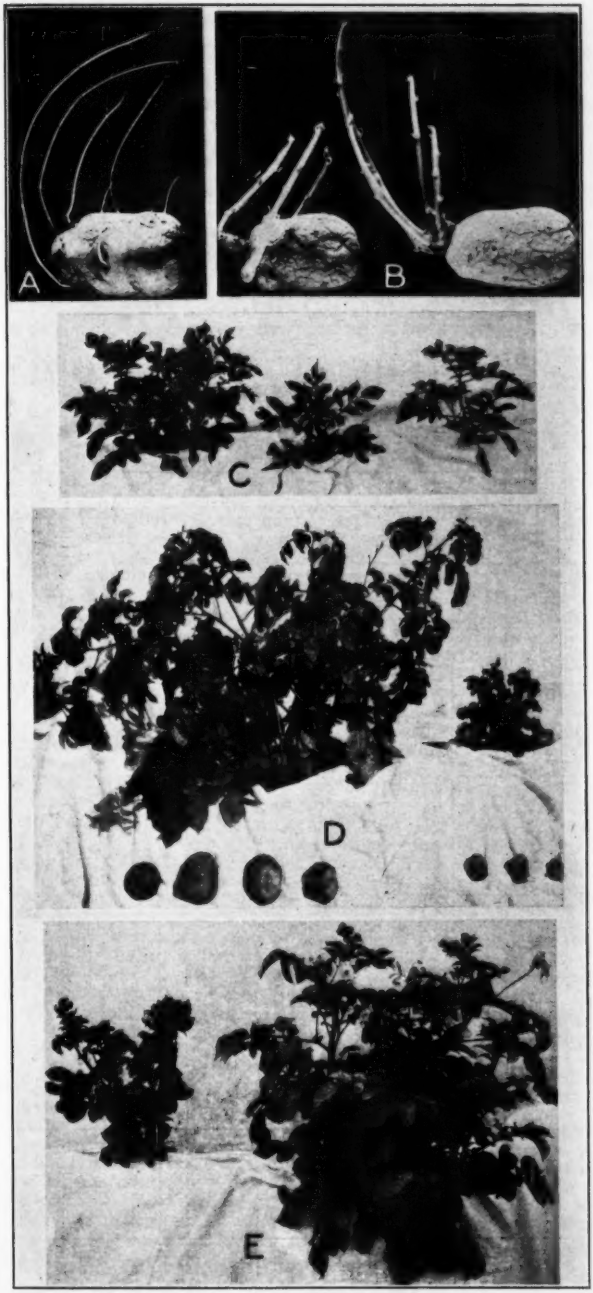
A.—Spindling-sprout tuber. Photographed on May 2, 1923. See Plate 5, B, for other tubers from the same lot, and Plate 5, C, for the plants grown from this tuber

B.—Tubers from the same lot as that shown in Plate 5, A, but without spindling sprouts. Photographed on May 2, 1923. See Plate 7, B and C for the plants grown from these tubers

C.—Plants grown from the tuber shown in Plate 5, A. Photographed on Aug. 11, 1923. Leaf-roll and mild mosaic are present

D.—Green Mountains of series 111 of Table II. The small hill represents the progeny of the inoculated hills and is infected with rugose mosaic and unmottled curly dwarf. The large hill represents the progeny of the sister hills of the inoculated hills, and is healthy. Photographed on Aug. 27, 1923. See Plate 4, D, for the source of inoculum

E.—Green Mountains of series 139 of Table II. The small hill represents the progeny of the inoculated hills and is infected with a leaf-rolling-mosaic spindle-tuber combination commonly known as curly dwarf (distinct from unmottled curly dwarf). The large hill represent the progeny of the sister hills of the inoculated hills, and is healthy. Photographed on Aug. 11, 1923. See Plate 4, E, for the source of inoculum. Compare with Plate 1, B, and Plate 4, B and C



(For explanatory legend see p 502)

TABLE III.—*Leaf mutilation inoculations in insect cages in 1922 and the progeny in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants					Uninoculated controls		
	Variety	Symptoms		Variety	1922 (3 hills treated in each case)		Progeny, 1923		1922. Number of hills infected or healthy and symptoms (3 hills examined in each case)	Progeny, 1923	
		1922	Progeny, 1923		Number of hills showing symptoms	Symptoms	Tuber units			Tuber units	
							Total number	Number diseased	Number of tuber units infected and symptoms	Total number	Number infected or healthy and symptoms
A-III*	Green Mountain.	Mild mosaic and spindle tuber.	Mild mosaic and spindle tuber.	Green Mountain.	3	Mild mosaic; both spindling and flat oblong tubers.	14	14	13 mild mosaic; 14 spindle tuber.	3 healthy	12	12 healthy.
B-III*	do	do	do	Irish Cobbler		No apparent mottling; spindling tubers in hills 1 and 2, normal and spindling tubers in hill 3.	14	14	14 spindle tuber. No apparent mottling.	2 spindle tuber	18	18 spindle tuber.
E-III	do	do	do	Green Mountain.	1	Mild mosaic and spindle tuber in hill 1. Hills 2 and 3 healthy.	17	12	2 mild mosaic; 12 spindle tuber.	3 healthy	24	24 healthy.
F-III	do	do	do	Irish Cobbler	1	No apparent mottling; spindling tubers in hill 2, normal and spindling in hill 3.	11	5	5 spindle tuber. No apparent mottling.	1 spindle tuber	17	10 spindle tuber.
G-III	do	do	do	Seedling 39374		No apparent mottling.	22	21	21 spindle tuber; 2 mild mosaic.	2 spindle tuber	14	9 spindle tuber.
H-III	do	do	do	Bliss Triumph.		do	13	7	5 spindle tuber; 2 mild mosaic.	3 healthy	16	16 healthy.
E-II	do	Mosaic dwarf	Mosaic dwarf	Green Mountain.	3	Rugose mosaic	16	16	8 rugose mosaic; 13 spindle tuber.	do	24	24 healthy.
F-II	do	do	do	Irish Cobbler		Apparently healthy	13	3	3 spindle tuber	1 spindle tuber	17	10 spindle tuber.
G-II	do	do	do	Seedling 39374	1	Spotting and streaking on hill 3.	24	17	4 spindle tuber; 13 no growth.	2 spindle tuber	14	9 spindle tuber.
H-II	do	do	do	Bliss Triumph.		Apparently healthy	19	13	13 spindle tuber; 2 rugose mosaic.	3 healthy	16	16 healthy.
E-I	Seedling 39374.	Streak	Badly dwarfed and spindle tuber.	Green Mountain.	3	do	16	10	10 spindle tuber	do	24	24 healthy.
F-I	do	do	do	Irish Cobbler	3	do	16	3	3 spindle tuber	1 spindle tuber	17	10 spindle tuber.
G-I	do	do	do	Seedling 39374	3	Streak and mottling	11	11	11 badly dwarfed	2 spindle tuber	14	9 spindle tuber.
H-I	do	do	do	Bliss Triumph	3	Apparently healthy	14	1	1 spindle tuber	3 healthy	16	16 healthy.

* Three inoculations at weekly intervals; one inoculation in remaining series.

tains and Bliss Triumphs, nothing in Irish Cobblers, and extreme dwarfing in the seedling. In both the mosaic dwarf and streak inoculation, therefore, the seedling appeared more susceptible to streak than the other varieties.

In 1922 in the control cage of the B series, and also in that of the F series, there was a healthy hill whose 1923 progeny developed spindle tuber. Each of these hills was grown in contact with a spindle tuber hill with aphids absent. Although other evidence on transmissibility by contact, as described later, is negative in the absence of insects, it should be borne in mind that even grafting, aphid, and leaf-mutilation inoculations often give negative results. It will also be pointed out later that such diseases can spread with neither aphids nor contact serving as the apparent means. This behavior of control hills emphasizes the need of careful selection of seed tubers for cage experiments and the need of repetition and duplication of inoculation experiments. The latter need is also brought out by the facts that streak was not contracted in the mosaic dwarf and streak inoculations by Green Mountains, although spindle tuber was contracted by the same plants from the same inoculum, that streak was contracted by the seedling from the same inoculum, and that under other conditions Green Mountains have contracted streak readily from the same type of inoculation. In brief, while streak in one certain inoculum did not infect Green Mountains, it did not follow that Green Mountains were resistant to streak in other inoculations, which indicates that with such diseases one inoculation is not conclusive, especially if the results are negative.

In series A–III, E–III, and E–I, a study of the data under "Progeny, 1923," shows that the infected plants contracted either spindle tuber or both spindle tuber and mild mosaic with no explanation needed for the isolation and greater amount of spindle tuber other than the apparent greater infectiousness of spindle tuber. However, in series E–II, of the same variety as the preceding series, there was still further separation of the viruses, some plants contracting rugose mosaic, some spindle tuber, and some both. The writers have no explanation to offer for this phenomenon, in which with all conditions apparently similar, a given inoculum affects inoculated plants in two or three different ways regarding the transmission of two diseases in the inoculum. Perplexity is increased by the fact, noted above, that streak in this inoculum did not affect any plants of these Green Mountain series.

INTERVARIETAL INOCULATIONS WITH APHIDS

Inoculations of Green Mountains and Irish Cobblers with rugose mosaic, leaf-roll, and spindle tuber were conducted with aphids in 1922. Instead of dispersing from diseased to healthy plants in the same cage as in 1921,[10] the aphids were transferred from diseased to healthy plants grown in separate cages. Approximately 50 to 200 aphids per cage were introduced when the plants had reached a height of 15 to 20 cm. After feeding from 12 to 20 days the aphids were killed with nicotine sulphate spray. The results of these inoculations for 1922, as well as for the progeny in 1923, are given in Table IV.

From the data presented in Table IV it is seen that inoculation was not followed by symptoms in 1922 or in 1923 with the exception of series J, which contracted leaf-roll. Spindle tuber and mosaic in one hill of series D–I, D–II, and D–III, apparently were introduced in the open field in 1921, since the uninoculated controls in the same tuber unit show the same symptoms. In series T–II spindle tuber infection may have resulted from aphids entering the cage through a hole found in the cage late in the season. The generally negative results in this experiment disclose that under certain conditions aphids do not transmit these diseases. As noted later in Tables VII and VIII, aphids in 1923 caused infection when transferred in conditions apparently like those of the inoculation series in Table IV. Whether these interesting variations are caused by differences in temperature, light, or other factors remains to be determined by further studies on the nature of these diseases and their transmission.

CURRENT-SEASON SYMPTOMS FROM INOCULATIONS IN 1923

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN THE OPEN FIELD

Leaf mutilation inoculations with different types of mosaic and with streak were made on plants of the Green Mountain variety in the open field in 1923. The effect of taking the inoculum from different parts of the

[10] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVI

TABLE IV.—*Inoculations with aphids in insect cages in 1922 and the progeny in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants			Uninoculated controls	
	Variety	Symptoms		Variety	Symptoms		1922 [a]	Progeny, 1923 [b]
		1922	Progeny, 1923		1922 [a]	Progeny, 1923 [b]		
C–I	Irish Cobbler	Spindle tuber	Spindle tuber	Green Mountain	3/3 healthy	20/20 healthy	3/3 healthy	24/24 healthy.
D–I	do	do	do	Irish Cobbler	1/3 spindle tuber	6/19 spindle tuber	1/3 spindle tuber	2/14 mosaic [c] and spindle tuber.
C–II	Seedling 39374	Rugose mosaic	No progeny	Green Mountain	3/3 healthy	20/20 healthy	3/3 healthy	24/24 healthy.
D–II	do	do	do	Irish Cobbler	1/3 spindle tuber	3/15 mosaic [c], 8/15 spindle tuber.	1/3 spindle tuber	2/14 mosaic [c] and spindle tuber.
C–III	Irish Cobbler	Spindle tuber	Spindle tuber and ruffled.	Green Mountain	3/3 healthy	12/12 healthy	3/3 healthy	24/24 healthy.
D–III	do	do	do	Irish Cobbler	1/3 spindle tuber	6/16 mosaic [c], 5/16 spindle tuber.	1/3 spindle tuber	2/14 mosaic [c] and spindle tuber.
T–I	Rosebush	Apparently healthy.	Apparently healthy.	Bliss Triumph	3/3 healthy	23/23 healthy		
T–II	do	do	do	do	1/3 spindle tuber	8/28 spindle tuber	6/6 healthy	6/6 healthy.
I	Irish Cobbler	Leaf-roll	Leaf-roll	Green Mountain	2/2 healthy	16/16 healthy		
J	do	do	do	Hebron	2/2 healthy	6/14 leaf-roll		
K	do	do	do	do	3/3 healthy	21/21 healthy		

[a] The two numbers in each fraction denote, respectively, the number of hills infected (or healthy) and the total number.
[b] The two numbers in each fraction denote, respectively, the number of tuber units infected (or healthy) and the total number.
[c] Mosaic in this variety was not marked, and was present in 1923 probably because of the generally favorable seasonal conditions for mosaic.

plant was tested with rugose mosaic in series 5 to 9. The remaining series represent inoculations comparable with similar series conducted in cages, the results of which will be noted later. The current-season effects of these inoculations are represented in Table V.

From the data on series 5 to 9 it is apparent that inoculum from either the entire shoots, the stems and petioles, or the leaflets alone, was more effective than that from roots or tubers; in fact, the very low percentage of infection that followed inoculations from roots or tubers may perhaps be due to field infection, although the uninoculated controls in these series remained healthy. The practically negative results from the inoculation with juice from the seed tubers is somewhat surprising, considering the facts that they perpetuate the disease and that grafting causes infection. These seed tubers were unplanted ones from the same lot as those from which the plants were grown that served as the source of inoculum for series 5 to 8; the whole lot came from diseased stock and all that were planted produced diseased plants. Inoculation with juice from the roots also gave practically negative results, although they were taken from the same plants as the shoots, leaflets, and stems and petioles, showing that not all parts of a plant contain the virus in a similar state of infectiousness. Infectiousness here seems to be correlated with the presence of chlorophyll. Not only were the colorless parts far less infective, but the inoculum from the leaflets was more infectious than that from the supporting parts of the plant, the latter infecting only 75 per cent of the plants inoculated instead of all of them. Also the inoculum from the leaflets was more infectious than that from the leaflets, petioles, and stems (aerial parts) combined, because, while infecting no more plants, still it infected them more completely by August 14, as is shown by Figure 1. As this figure indicates, of the 20 plants infected from the leaflets (in section W–7, row 2), 16 were obviously diseased in the upper leaves of all the shoots by August 14, while of the 20 plants infected from the shoots (in row 4), only 9 were thus affected. The inoculum was used from an uncovered dish; possibly when present chlorophyll prevented a sterilization of the inoculum by light.

Inoculations with mild mosaic (in series 14 of Table V) induced the appearance of no current-season symp-

TABLE V.—*Leaf mutilation inoculations of Green Mountains in the open field in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants			Uninoculated controls	
	Variety	Symptoms		Total number	Number infected	Symptoms	Number of hills	Symptoms
		1922	Progeny, 1923					
5	Green Mountain.	Rugose mosaic.	Rugose mosaic, roots.	10	1	Rugose mosaic	30	Healthy.
6	do	do	Rugose mosaic, leaflets.	20	20	do	20	Do.
7	do	do	Rugose mosaic, stems and petioles.	20	15	do	20	18 hills healthy.
8	do	do	Rugose mosaic, shoots.	20	20	do	20	Healthy.
9	do	do	Seed tubers	16	2	do	24	Do.
10	do	Healthy	Healthy	17	0	Healthy	19	Do.
14	do	Mild mosaic.	Mild mosaic	10	0	Apparently healthy	10	Do.
16	do	Leaf-rolling mosaic.	Leaf-rolling mosaic.	10	10	Leaf-rolling mosaic.	10	Do.
18	do	Rugose mosaic.	Rugose mosaic	5	5	Rugose mosaic	15	Do.
20	do	Streak	Streak and slight mottling.	5	1	Streak	15	Do.
22	do	Healthy	Healthy	10	0	Healthy	30	Do.
29	Irish Cobbler.		Mild mosaic (?)	10	0	do	30	Do.
30	do		Rugose mosaic with streaking.	5	5	Rugose mosaic including streaking, spotting and burning.	15	Do.
31	do		do	10	8	Rugose mosaic in 6 hills. Rugose mosaic including streaking, spotting and burning in 2 hills.	30	Do.

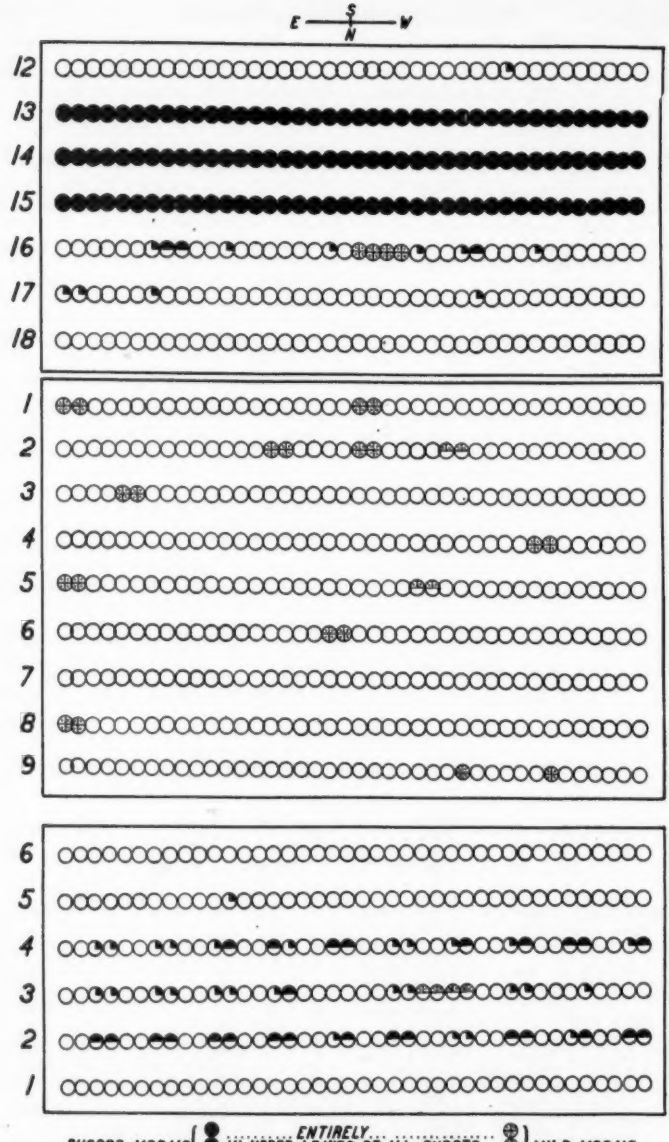


FIG. 1.—Diagrammatic representation of the results of inoculation with rugose mosaic and of proximity in 1923 to plants with this disease. Each circle represents a hill (drawn to scale as of one foot in diameter of each circle), with disease indicated as explained in the legend. See also Plate 9, A. In Section W–1, planted on May 15, and observed on July 31, row 12 was planted with control healthy stock, rows 13 to 15 with seed from rugose mosaic plants, and rows 16 to 18 as for row 12. Section W–6 was planted on May 19, as a control plot, from the same stock as the control rows in Section W–1, and was observed on Aug. 14. Section W–7 was planted on June 19 and 22 with the same control stock as above, was inoculated on July 14, and was observed on August 14. In this section, hills of row 1 were inoculated with juice from the roots of rugose mosaic plants, row 2 from the leaflets, and row 3 from the stems and petioles, row 4 from the shoots (stems and leaves), row 5 from the seed tubers, and row 6 with tap water. On this section each row consists of 10 four-hill tuber units, and one or two hills in each tuber unit were inoculated, these being the two right-hand or west hills in each tuber unit in rows 2, 3, and 4. In section W–6, each row consists of 20 two-hill tuber units. For further discussion see the text on pp. 507 and 509

toms, which is in marked contrast to the complete positive results obtained with leaf-rolling and rugose mosaic in the open (series 8, 16, and 18) and with mild mosaic in cages (described later). In series 29, a mild type of mottling, tentatively called a mosaic, on Irish Cobblers likewise failed to produce current-season symptoms on inoculated Green Mountains. Negative results with a similar type of so-called mosaic on Irish Cobblers were obtained with stalk grafts on 12 Green Mountain shoots in three separate tuber units in cages. This seems to suggest that the mottling on the Irish Cobblers is not infectious, but to verify this conclusion similar negative reactions in the second generation are essential. In any case this mottling on Irish Cobblers appears distinct from rugose mosaic, as described previously and as disclosed by the positive current-season reactions in series 30 and 31; in these series almost 100 per cent infection resulted from rugose mosaic inoculations performed under the same conditions as for the mottled Irish Cobbler inoculation.

In connection with the use of juice from seed tubers as described above, the results of tests of knife transmission may be noted. The use of the same knife to cut alternately diseased and healthy tubers at planting time, with 10 healthy tubers thus inoculated with each disease, did not result in transmission. Each of the four types of mosaic, spindle tuber, unmottled curly dwarf, leaf-roll, and several combinations were tested.

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN INSECT CAGES

In order to determine the comparative reactions of the Green Mountain, Irish Cobbler, and Rural New Yorker varieties to mild, leaf-rolling, and rugose mosaic, and of Green Mountains to streak, leaf mutilation inoculations were performed in insect cages in 1923. Inoculations with mosaic were made on the same date, when the height of the plants varied from 7 to 30 cm. Diseased Green Mountains served as the sources of inoculum. The method of planting three hills in each cage, from three tuber units, respectively, resembled that previously described for cage experiments. The current-season results of these inoculations are recorded in Table VI.

From the results presented in Table VI it is quite apparent that rugose mosaic in series A–3, B–3, and C–3 produced complete infection in the three varieties. Inoculations with mild and leaf-rolling mosaic were equally effective on Green Mountains (pl. 6, A) but not apparently so on Irish Cobblers and Rural New Yorkers.[11] It is also noteworthy that the first symptoms of mild mosaic, leaf-rolling mosaic, and rugose mosaic appeared, respectively, 32, 25, and 18 days after inoculation, indicating different incubation periods for these mosaic types. The absence of current-season symptoms with mild mosaic on Irish Cobblers and Rural New Yorkers suggests either differences in varietal susceptibility or varietal modification of symptoms of this disease. Reactions resulting from aphid inoculations have been recorded.[12] Table VII in this paper gives the data from later work. Streak was transmitted to Green Mountains resulting in the characteristic current-season symptoms (pl. 2, B).

INTERVARIETAL INOCULATIONS WITH APHIDS

For comparison with the leaf-mutilation inoculations described in the preceding section of this paper, inoculations were performed with aphids in insect cages in the field in 1923. Aphids, *Macrosiphum solanifolii* Ashm., obtained from rose bushes, were transferred to healthy Bliss Triumphs and then to Green Mountains affected respectively with different types of mosaic. After feeding for about 10 days on diseased plants, approximately 50 to 100 aphids per hill were introduced to healthy caged plants varying in height from 7 to 30 cm. After feeding from 12 to 13 days on the inoculated plants the aphids were killed with nicotine sulphate spray. Current-season reactions are recorded in Table VII.

As is shown in Table VII, of the three varieties, Green Mountain, Irish Cobbler, and Rural New Yorker, that were tested, only the Green Mountain showed positive results with mild and leaf-rolling mosaic. All three varieties showed slight symptoms resulting from inoculations with rugose mosaic. However, the reactions to rugose mosaic were not so pronounced as those with the leaf mutilation inoculations denoted in Table VI. No current-season symp-

[11] Since the submittal of this manuscript observations on the second generation disclosed mild mosaic on Rural New Yorker and leaf-rolling mosaic on Irish Cobbler and Rural New Yorker.

[12] SCHULTZ, E. S., and Folsom, D. Op. cit. p. 65–84.

TABLE VI.—*Leaf mutilation inoculations in insect cages in 1923*

Inoculation series No.	Symptoms in source of inoculum [a]		Inoculated plants (3 hills in each case)					Uninoculated controls	
	1922	Progeny, 1923	Variety	Height when inoculated	Date of inoculation	Date of first symptoms	Number of hills infected and symptoms	Number of hills	Number of hills infected and symptoms
				Cm.					
A–1	Mild mosaic	Mild mosaic	Green Mountain	15–25	July 16	Aug. 17	3 mild mosaic	3	3 healthy.
B–1	do	do	Irish Cobbler	17	do		3 apparently healthy	3	Do.
C–1	do	do	Rural New Yorker	15–25	do		do	3	Do.
A–2	Leaf-rolling mosaic	Leaf-rolling mosaic	Green Mountain	20		Aug. 10	3 leaf-rolling mosaic	3	Do.
B–2	do	do	Irish Cobbler	10–15	July 16	Aug. 15	1 slight mottling on axillary shoots	3	Do.
C–2	do	do	Rural New Yorker	7–30	do		3 apparently healthy	3	Do.
A–3	Rugose mosaic	Rugose mosaic	Green Mountain	17	do	Aug. 3	3 rugose mosaic	3	Do.
B–3	do	do	Irish Cobbler	15	do	Aug. 15	do	3	Do.
C–3	do	do	Rural New Yorker	7	do	do	do	3	Do.
G–9	Streak	Streak and slight mottling	Green Mountain	15–17	July 17		3 apparently healthy	3	Do.
G–10	do	do	do	15–17	do	Aug. 15	2 streak without mottling	3	Do.

[a] All sources were plants of the Green Mountain variety.

TABLE VII.—*Inoculations with aphids in 1923*

Inoculation series No.	Symptons in source of inoculum [a]		Inoculated plants (3 hills in each case)					Uninoculated controls	
	1922	Progeny, 1923	Variety	Height when inoculated	Aphids: Approximate number per hill	Aphids: Feeding period	Number of hills infected and symptoms	Hills	Symptoms
				Cm.		*Days*			
A–5	Mild mosaic	Mild mosaic	Green Mountain.	17	100+	13	2 mild mosaic	3	Healthy.
B–5	do	do	Irish Cobbler	15	100±	13	3 apparently healthy	3	Do.
C–5	do	do	Rural New Yorker.	7–15	100±	13	do	3	Do.
A–6	Leaf-rolling mosaic	Leaf-rolling mosaic	Green Mountain.	17–30	50±	13	3 leaf-rolling mosaic	3	Do.
B–6	do	do	Irish Cobbler	15–25	50±	12	3 apparently healthy	3	Do.
C–6	do	do	Rural New Yorker.	7–15	50±	12	do	3	Do.
A–7	Rugose mosaic	Rugose mosaic	Green Mountain.	20–25	50±	12	1 slight rugose mosaic	3	Do.
B–7	do	do	Irish Cobbler	17	50±	12	1 slight mottling on young shoots	3	Do.
C–7	do	do	Rural New Yorker.	10–15	50±	12	1 streaking and rugosity of foliage	3	Do.
G–7	Streak	Streak and slight mottling	Green Mountain.	16	50+	13	3 apparently healthy	3	Do.
D–1	Unmottled curly dwarf	Unmottled curly dwarf	do	17	50+	13	3 one tuber per hill somewhat spindling.	3	Do.
E–1	do	do	Irish Cobbler	17	50+	13	2 one tuber per hill spindling	3	Do.
F–1	do	do	Rural New Yorker.	10–15	50+	13	1 one tuber somewhat spindling	3	Do.

[a] All sources were plants of the Green Mountain variety.

toms appeared on the foliage of plants inoculated with unmottled curly dwarf in series D–1, E–1, and F–1; however, some tubers in this series were more or less spindling. The negative results obtained with aphid inoculations of Irish Cobblers and Rural New Yorkers with mild mosaic apparently suggest either varietal modification of symptoms or differences in susceptibility to this disease. Similar reactions were described in the preceding section of this paper. The streak inoculation with aphids was not followed by symptoms as with leaf-mutilation inoculation (see Table VI).[13]

Some comment may appropriately be made here upon the still frequently repeated statement that mosaic becomes worse as the number of years increases since it has infected a given stock. The statement in question does not refer so much to the often demonstrated fact that the percentage of mosaic may increase in a stock until the latter is entirely diseased, but rather implies that mosaic is mild at first, then medium, and finally severe. According to this theory it is hard to explain the results described in this and the previous sections of the present paper, where inoculum introduced by means of leaf mutilation or aphids did not invariably produce mild mosaic as the first-year symptoms, but instead produced respectively the same symptoms as were observed on the plants from which the inoculum was obtained (pl. 6, A and B). It seems preferable to maintain that there are several types of mosaic, each retaining its characteristic symptoms indefinitely under the same environmental and varietal conditions, while being perpetuated by the tubers or even when transmitted from one plant to another.

In this connection it may be added that the mild mosaic plants from which the first Green Mountain series in Table VI and in Table VII were infected, were known to be of the eighth generation of a strain originating from mild mosaic hills selected in 1916. During that time the stage of mosaic had not become worse (pl. 7, A), although of course there was some variation with the seasons and some other progeny of the original selections had acquired additional diseases that made them appear to have curly dwarf, bad mosaic, and mosaic dwarf.

It may also be suggested that in applying these methods to potato degeneration problems in other regions, it will be found helpful to use insect cages with openings in the top (pl. 8, A) this feature facilitating the proper care of a large number of cages (pl. 8, B).

INTERVARIETAL DISEASE-COMBINATION INOCULATIONS WITH APHIDS

Combination inoculations with mild, leaf-rolling, rugose, and crinkle mosaic, leaf-roll, and spindle tuber, were made on Green Mountains, Irish Cobblers, and Rural New Yorkers in insect cages in the field. Aphids were introduced into each cage from plants obtained from two sources and with respectively different diseases. The methods of inoculation, including the aphid transfers, resembled those described in the previous section of this paper. In each series aphid transfers were made from the two sources on the same date with the exception of series G–2, G–3, and G–5, in which aphids from leaf-rolling mosaic plants were introduced to healthy plants 13, 7, and 12 days, respectively, before the aphids from the other disease in the combination were transferred.

Approximately 50 to 100 aphids were introduced to each of the three hills in a cage when the height of the plants varied from 7 to 40 cm. The current-season results of these inoculations are indicated in Table VIII.

The data presented in Table VIII show that combinations of rugose mosaic and spindle tuber became apparent in series D–3 and E–3; of crinkle mosaic and spindle tuber in series G–1; and of leaf-rolling mosaic and leaf-roll in series G–3.

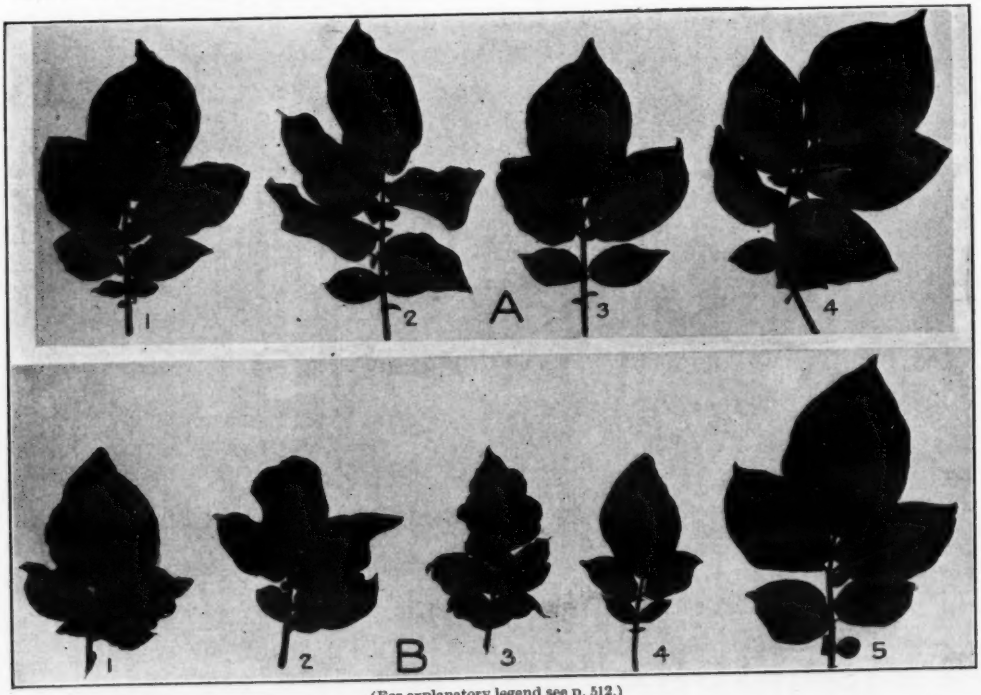
It is noteworthy that leaf-roll in the leaf-rolling mosaic and leaf-roll combination retained the characteristic symptoms of leaf-roll with leaf-rolling

[13] Since the submittal of this manuscript, observation on the second-generation plants disclosed typical second-generation streak symptoms. Aphid transmission of streak has also been reported by Atanasoff (p. 32, Rpt. Internat. Confer. Phytopath. Econ. Entom. Holland, 1923).

EXPLANATORY LEGEND FOR PLATE 6

A.—Representative leaves from 4 hills of the same Green Mountain tuber unit grown in cages of Table VI and infected with the leaf-mutilation method as follows: 1, cage A–3, rugose mosaic; 2, cage A–2, leaf-rolling mosaic; 3, cage A–1, mild mosaic; 4, cage A–4 (control to others), healthy. Photographed on Sept. 6, 1923. Compare with Plate 6, B

B.—Representative leaves from 5 Green Mountain hills inoculated in insect cages with aphids and infected with rugose mosaic (1), leaf-rolling mosaic (2), crinkle mosaic (3), and mild mosaic (4), and healthy (5). Photographed on Sept. 6, 1923. Compare with Plate 6, A



(For explanatory legend see p. 512.)

mosaic symptoms added, demonstrating a distinction between these two diseases. Similar distinctions appeared in the other series. Usually more marked dwarfing resulted from these combinations than from the single diseases.

CONTACT INOCULATIONS IN THE GREENHOUSE

In order to ascertain whether the mere contact of the seed pieces, roots, and shoots of spindle-tuber plants with those of healthy plants would produce infection, seed pieces from diseased and healthy plants were planted together in 10-inch pots in the Washington greenhouses during the winter of 1922–23. The healthy plants were of three varieties, Green Mountain, Irish Cobbler, and Bliss Triumph. Each variety was represented by 14 healthy tubers which were split in two. One-half of each healthy Green Mountain and Irish Cobbler tuber was planted in contact with a spindle tuber Bliss Triumph seed piece, while a half of each healthy Bliss Triumph tuber was planted in contact with a spindle tuber Green Mountain seed piece. Here contact of freshly cut surfaces was not intimate, as in tuber grafting. The other healthy tuber halves served as controls and were planted in 8-inch pots. From the time of planting, January 5, 1923, until harvest, April 12, 1923, the forty-two 10-inch pots were covered with aphid-proof cages, while the control plants in 8-inch pots remained uncaged in the same greenhouse.

Three observations during this experiment did not disclose any insects on the caged plants. On account of seed piece decay six of the healthy Green Mountains in 10-inch pots failed to produce tuber progeny.

At harvest all tuber progeny from the contact healthy plants as well as from the control plants appeared normal, while the tuber progeny from the spindle tuber plants which had attained sufficient size for diagnosis were spindle-shaped.

The second generation Irish Cobbler and Bliss Triumph plants were grown in the open field at Presque Isle, Me., and those of the Green Mountain variety in the Washington, D. C., greenhouse. In this generation 31 Irish Cobbler tuber units, representing 13 of the original contact half tubers (one half tuber being without tuber progeny), as well as the respective controls, were healthy. Spindle tuber progeny resulted from all the original spindle tuber plants. In the second generation 29 Bliss Triumph tuber units, representing 11 contact half tubers of the first generation and their respective controls, remained free from spindle tuber. One other contact half tuber and its respective control produced spindle tuber plants (in the first generation in the greenhouse) possibly as a result of late season field infection the previous season. Two contact half tubers failed to produce tubers in the first generation because of seed-piece decay. Likewise, in the second generation 22 Green Mountain tuber units, representing 11 half tubers of the first generation and the respective controls, failed to produce spindle tuber progeny.

These observations confirm similar ones on the contact of mosaic and leaf-roll plants with insects excluded, where negative evidence was obtained.[14]

A similar experiment but without insect cages, was performed in the Orono, Me., greenhouse at about the same time. Here Bliss Triumph tubers that were healthy or with mosaic of several types, leaf-roll, or spindle tuber were planted in steam-sterilized soil, each in a 10-inch pot with a healthy Green Mountan tuber. There were two similar series, W and C, which were placed in separate rooms, the former in a warmer place than the latter. Planting was done in the second week of December, 1922. White flies (*Asterochiton vaporariorum*) appeared in January on other plants in the same greenhouse and were cyanide fumigated on January 12 and 18. All but one of the Bliss Triumph plants had emerged from the soil by January 22, and the last one and also the Green Mountains had emerged by February 8. White flies were present again, on the C series this time, in February and later in April, and were cyanided each time. Frequent observations disclosed no aphids at any time, in this

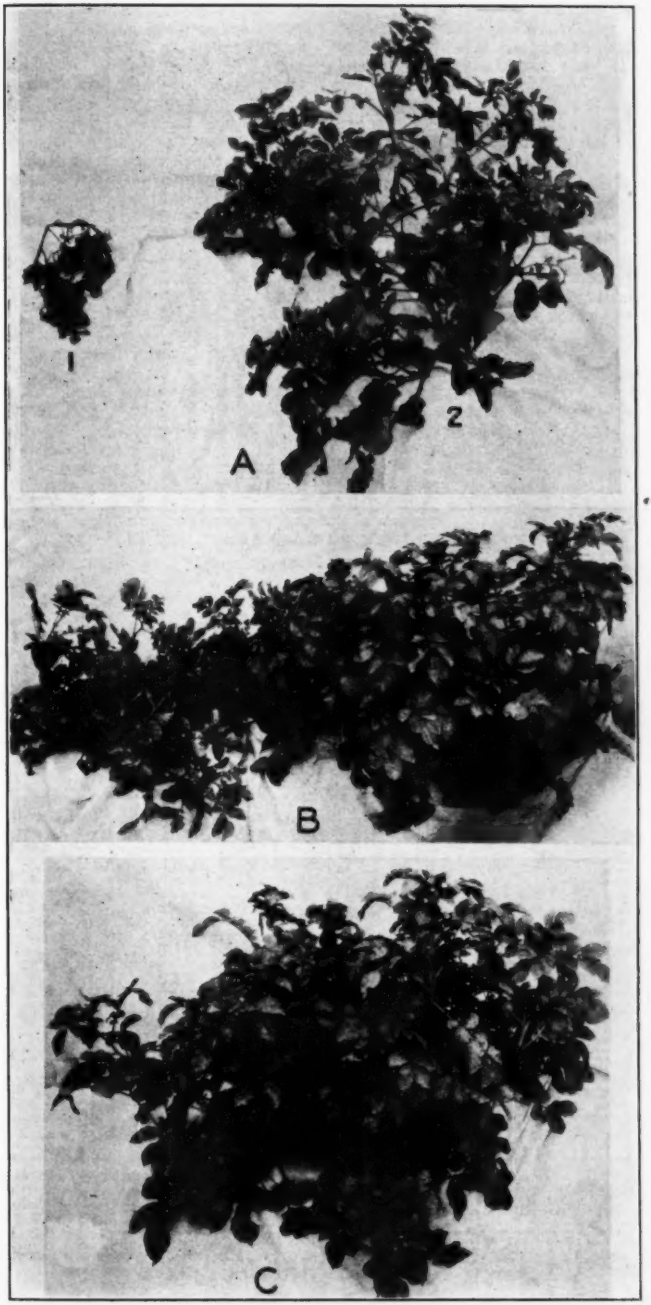
[14] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 46–48, 54.

EXPLANATORY LEGEND FOR PLATE 7

A.—Mild mosaic (2) in a Green Mountain hill of a strain known to be entirely mosaic from 1916 to 1923, inclusive, compared with a rugose mosaic hill of the same variety (1). Photographed on Sept. 10, 1923, after the death of the rugose mosaic vine

B.—Plants grown from the right-hand tuber of Plate 5, B. Photographed on Aug. 11, 1923. Mi mosaic is present. Compare with Plate 5, C

C.—Plants grown from the left-hand tuber of Plate 5, B. Otherwise as for Plate 7, B



(For explanatory legend see p. 514)

TABLE VIII.—*Disease combination inoculations with aphids in 1923*

Inoculation series No.	Symptoms in sources of inoculum [1]		Inoculated plants (3 hills in each case)					Controls	
	1922	Progeny, 1923	Variety	Height when inoculated	Aphids: Approximate number per hill	Aphids: Feeding period	Number of hills infected and symptoms	Hills	Symptoms
				Cm.		*Days*			
D-2	Mild mosaic	Mild mosaic	Green Mountain	15	50+	13	2 one tuber per hill somewhat spindling	3	Healthy.
	Spindle tuber	Spindle tuber		15	50+	13			
D-3	Rugose mosaic	Rugose mosaic	....do	15–20	50±	13	3 rugose mosaic with streaking in 1 hill	3	Do.
	Spindle tuber	Spindle tuber		15–20	50±	13	2 one tuber per hill somewhat pear shaped	3	
E-2	Mild mosaic	Mild mosaic	Irish Cobbler	15	50+	14	3 one tuber per hill somewhat spindling	3	Do.
	Spindle tuber	Spindle tuber		12–17	50±	13			
E-3	Rugose mosaic	Rugose mosaic	....do	12–17	50±	13	2 rugose mosaic and 1 tuber per hill somewhat spindling. Third hill with two tubers somewhat spindling.	3	Do.
	Spindle tuber	Spindle tuber		12–17	50±	13			
F-2	Mild mosaic	Mild mosaic	Rural New Yorker	7–15	50+	14	1 three tubers spindling	3	Do.
	Spindle tuber	Spindle tuber		7–15	50±	13			
F-3	Rugose mosaic	Rugose mosaic	....do	15–20	50±	13	One tuber in 1 hill and 2 tubers in another hill spindling	3	Do.
	Spindle tuber	Spindle tuber		15–20	50±	13			
G-1	Crinkle mosaic [2]	Crinkle mosaic [2]	Green Mountain	15	50+	13	3 crinkle mosaic. Two tubers in hill 1 and one tuber in hill 2 somewhat spindling	3	Do.
	Spindle tuber	Spindle tuber		15	50+	13			
G-2	....do	....do	....do	40	50+	13	3 leaf-rolling mosaic	3	Do.
	Leaf-rolling mosaic	Leaf-rolling mosaic		13–20	50+	13			
G-3	....do	....do	....do	13–15	50+	13	3 leaf-rolling mosaic and leaf-roll	3	Do.
	Leaf-roll	Leaf-roll		30–35	100+	13		3	Do.
G-5	Rugose mosaic	Rugose mosaic	....do	30–35	100+	13	1 leaf-rolling mosaic and possibly rugose mosaic	3	Do.
	Leaf-rolling mosaic	Leaf-rolling mosaic		15–20	50+	17			
G-6	Mild mosaic	Mild mosaic	....do	17–35	50+	13	1 mild mosaic	3	Do.
	Streak	Streak		17–35	50+	13			

[1] All of the Green Mountain variety.
[2] Apparently a fourth type of mosaic; probably not identical with the "crinkle" of other writers.

respect duplicating conditions of the preceding winter in this greenhouse. Thrips were present as usual, being more abundant where the W series was grown. The plants in these series were observed frequently and the tubers when dug, on April 4, were also examined. Conditions for spindle tuber diagnosis were considered somewhat unfavorable. The tubers were kept in cold storage during the summer. The plants of the second generation were grown in the latter months of 1923, one only from each tuber. The mosaic and leaf-roll observed in the two generations designated, respectively, because of the time of planting as those of 1922 and 1923, although both were grown in 1923, are given in Table IX.

It will be seen from this table that about two-thirds of the Bliss Triumphs were mosaic, with one healthy 1922 hill producing mosaic progeny. Of the Green Mountains, about two-thirds were grown in 1922 in root contact with Bliss Triumph mosaic hills, that is, 24 of a total of 37. Of the 24, 5 produced mosaic progeny; and of the 37, 7 produced mosaic progeny. The difference of 2 represents two plants in root-contact with leaf-roll Bliss Triumphs. None of the Green Mountains contracted leaf-roll. Evidently conditions were favorable for the spread of mosaic both with and without root contact, but not for the spread of leaf-roll. The abundance of mosaic plants and the arrangement of the plants made leaf contact with mosaic plants possible for the two Green Mountain hills that were not in root contact and that became mosaic. It will be noted that only one tuber (of only one hill, of course) became mosaic in the C series, where white flies were present. The greater number of infections occurring in the W series leads to the suggestion of the possibility of transmission by thrips;[15] this difference, together with the fact that some of the 1922 hills had both diseased and healthy progeny, also indicates that the infection of the Green Mountains occurred in the greenhouse and was not present in the tubers when they were planted in 1922. Inasmuch as thrips can be controlled readily in experiments and have no practical importance as to potatoes, no tests are planned to test this suggestion about their transmitting potato mosaic. It remains clear, however, that mosaic can spread in the absence both of aphids and of artificial inoculation.

TABLE IX.—*Effect of proximity to disease in the absence of aphids*

	Number of hills, 1922			Number of hill lots, 1923 [a]			Number of hills, 1923 [b]		
	Total	Mosaic	Leaf-roll	Total	Mosaic	Leaf-roll	Total	Mosaic	Leaf-roll
W—Green Mountain series:									
In root contact with Bliss Triumphs in 1922	20	0	0	20	[c] 6	0	73	18	0
In root contact with mosaic Bliss Triumphs 1922	14	0	0	14	[c] 5	0	51	14	0
In root contact with leaf-roll Bliss Triumphs [d] in 1922	2	0	0	2	1	0	8	4	0
W—Bliss Triumph series	20	14	[d] 2	20	14	2	100	69	8
C—Green Mountain series:									
In root contact with Bliss Triumphs in 1922	17	0	0	17	[c] 1	0	65	1	0
In root contact with mosaic Bliss Triumphs in 1922	10	0	0	10	0	0	41	0	0
In root contact with leaf-roll Bliss Triumphs in 1922	2	0	0	2	[c] 1	0	9	1	0
C—Bliss Triumph series	17	10	[d] 2	17	11	2	85	54	4
W and C—Green Mountain series:									
In root contact with Bliss Triumphs in 1922	37	0	0	37	[c] 7	0	138	19	0
In root contact with mosaic Bliss Triumphs in 1922	24	0	0	24	[c] 5	0	92	14	0
In root contact with leaf-roll Bliss Triumphs in 1922	4	0	0	4	[c] 2	0	17	5	0
W and C—Bliss Triumph series	37	24	[d] 4	37	25	4	185	123	12

[a] Each from a hill of 1922.
[b] Each hill grown from a different tuber (produced in 1922).
[c] Including those partly diseased.
[d] None of the leaf-roll Bliss Triumphs were also mosaic.
[e] Partly diseased.

[15] Thrips are suspected as a carrier of a mosaic of Eucharis lilies. WHETZEL, H. H. REPORT OF THE PLANT PATHOLOGIST FOR THE PERIOD JANUARY 1ST TO MAY 31ST, 1922. Bermuda Rpts. Bd. and Dept. Agr. 1922: 29, 30-31, 1923.

INOCULATIONS WITH APHIS ABBREVIATA

Three genera of aphids have been found on potatoes in northeastern Maine, represented by *Macrosiphum solanifolii* Ashmead, *Myzus persicae* Sulz., and *Aphis abbreviata* Patch.[16] It is well known from previous studies by the writers and others that *Macrosiphum solanifolii* and *Myzus persicae* transmit virus diseases of the potato and other cultivated plants.[17] *Aphis abbreviata* feeds very generally on the lower leaves of the potato. By color, size, and shape it can be distinguished readily from the other species when on potatoes. Since *Aphis abbreviata* has been observed on potato plants barely 12 cm. high, it is apparent that it may sometimes be present before it is possible to eliminate diseased plants even in the first roguing.

During 1923 when *Aphis abbreviata* appeared to be especially numerous in comparison with previous years and in comparison with the other species, inoculation experiments with this species were begun. Since the overwintering host of *Aphis abbreviata* was not yet definitely known, colonies from potato foliage were used for inoculations. This procedure resulted unfortunately in the introduction of a few individuals of *Macrosiphum solanifolii*, so that the positive results with *Aphis abbreviata* in field cages in the first test were not conclusive.

However, later in the season pure *Aphis abbreviata* colonies were secured from E. M. Patch, as well as from isolation cultures in field insect cages. After these aphids colonized on mild mosaic caged Green Mountain plants for a few weeks, about 300 of them were transferred to each of three caged healthy Green Mountain hills when the plants were about 30 cm. high. At the same time transfers from the same colony were made to sprouts of three healthy Green Mountain half tubers. The lateness of the season apparently prevented sufficient additional foliage growth for the appearance of current-season symptoms in the cages. However, in the greenhouse second generation plants from a tuber from each of the three caged hills showed mild mosaic throughout the plants. The control plants from the three sister tuber progeny were healthy. Of the plants grown from three sprouted half tubers inoculated with *Aphis abbreviata* from mild mosaic Green Mountain plants, two developed mild mosaic when about 20 cm. high, while the corresponding half-tuber controls, from the same tubers as the inoculated plants, remained healthy.

NATURAL SPREAD OF MOSAIC WITH CURRENT-SEASON SYMPTOMS

In 1923 colonies of apterous *Aphis abbreviata* appeared somewhat earlier and in greater numbers than those of the other species on the disease-propagating plot, the first colonies being observed when the potato plants were scarcely 12 cm. above ground. Following this, current-season rugose mosaic symptoms (pl. 9, A) appeared in healthy stock in rows near or adjacent to rows of diseased plants. This is shown diagrammatically in Figure 1. In section W–1, 60 per cent of the four-hill tuber units and 23 per cent of the hills in the first row (16) on the north of three rugose mosaic rows (13–15) were rugose mosaic in the upper leaves of part or of all of the shoots by July 31. The disease also spread to the first row on the south and to the second row on the north with similar current-season effects but to a less extent. The difference between the two adjoining rows on the north and south indicates transmission by insects rather than by root contact.

[16] PATCH, E. M. THE BUCKTHORN APHID. Me. Agr. Exp. Sta. Bul. 317: 29-52, illus. 1924.
[17] SCHULTZ, E. S., and FOLSOM, D. Op. cit.

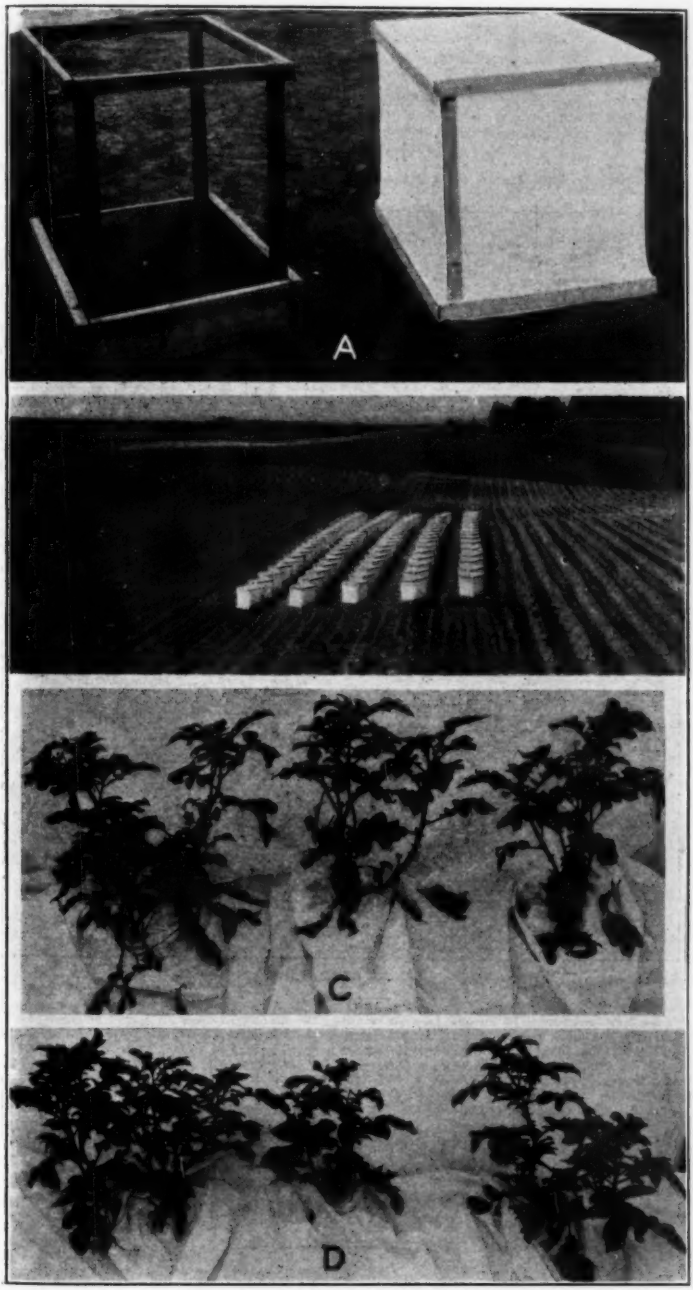
EXPLANATORY LEGEND FOR PLATE 8

A.—Frame and completed insect cage suitable for 3 potato hills. The cloth is in two pieces, one covering the four sides and the other across the top with an overlap in the middle and a slit in the center cut at right angles to the overlap

B.—Insect cages used in 1923, each covering 3 potato hills. The top opening (A) facilitates the proper care of a large number of cages. The arrangement shown permits the inclusion of the maximum number of hills possible with the available light

C.—Green Mountain tuber unit showing delayed symptoms of rugose mosaic after perpetuation of the disease by the seed tuber. The parent hill was partly diseased. This tuber unit appeared healthy on July 6, 1923, when other tuber units were obviously rugose mosaic (D), but the apparently healthy leaves developed spotting, streaking, and leaf-dropping, and the upper leaves showed typical rugose mosaic symptoms throughout by July 30 when this photograph was taken

D.—Green Mountain tuber unit completely rugose mosaic. This tuber unit was planted next to that of C in the same row, and was evidently diseased on July 6 when the other was still healthy. Note the greater dwarfing and the absence of leaf-dropping. Photographed on July 30, 1923, to the same scale as for D



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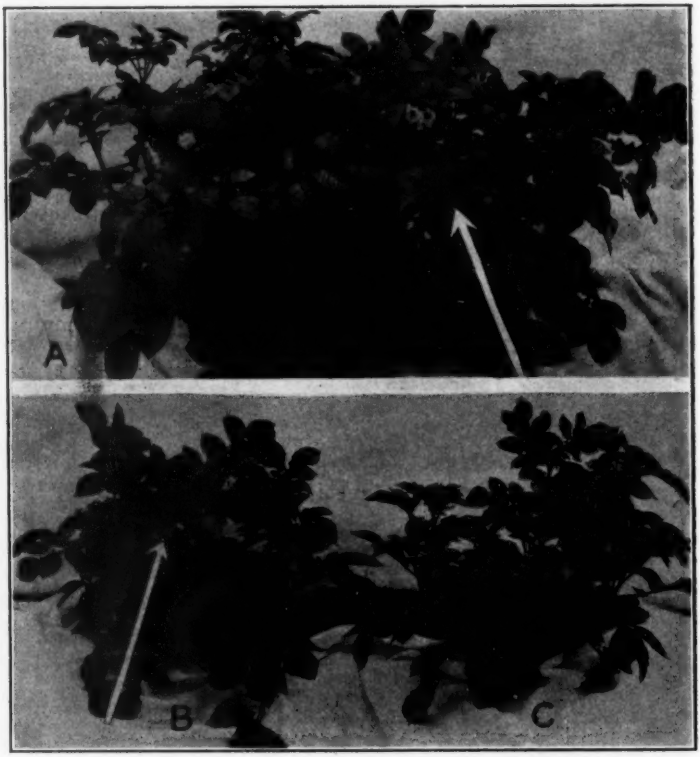


A.—On right, Green Mountain plant healthy except for current-season symptoms of rugose mosaic, indicated by the arrow. This is the twelfth hill from the east end of row 16 in section W-1 (fig. 1), as viewed from the south side. On left, healthy Green Mountain plant (thirteenth hill). Photographed on July 30, 1923. See Plate 8, C and D, for rugose mosaic from the seed tuber

B and C.—On right, Green Mountain plant with leaf-rolling mosaic perpetuated by the seed piece. On left, Green Mountain plant healthy except for current-season symptoms of leaf-rolling mosaic, indicated by arrow. Photographed on July 30, 1923. For a healthy control see Plate 9, A, on left

This is also indicated by the spread to the second row. There was no shoot contact early enough to permit the possibility of transmission by such means. That the disease spread in the plot in 1923 and was not perpetuated in the tubers from 1922 is indicated by the difference between the first and second rows on the north and by the absence of any rugose mosaic in the third row on the north and also nine rows (fig. 1, sec. W–6) planted elsewhere with tubers from the same healthy stock. Furthermore, other parts of the same healthy stock that were inoculated artificially as early in 1923 as diseased plants were available became diseased at about the same time and in the same way, except with a higher percentage of incidence.

Whether rugose mosaic was spread here by means of flea beetles (*Epitrix cucumeris* Harris), tarnished plant bugs (*Lygus pratensis* L.), or aphids, is open to question. *Aphis abbreviata* was observed here at the time of securing the inoculum for the artificial inoculations. However, roguing in another seed-plot, somewhat isolated, before any aphids were present and while flea beetles and tarnished plant bugs were present, did not result in the elimination of mosaic of the mild type. Transmission by flea beetles (*Psylloides affinis*), capsid bugs (*Calocoris bipunctatus*), and jassids (*Typhlocyba Ulmi*) has been demonstrated for leaf-roll by Murphy.[18]

In the same disease propagation plot there was also some spread of leaf-rolling mosaic, but only to the adjoining healthy row on the north, with the symptoms (pl. 9, B and C) appearing later than for rugose mosaic, in accordance with the results described previously, showing that rugose mosaic, leaf-rolling mosaic, and mild mosaic have progressively longer incubation periods. Contrary to what might be expected from these differences in the length of the incubation period, experience has shown that rugose mosaic, leaf-rolling mosaic, and mild mosaic are progressively more easily transmitted in the field from diseased to healthy plants (see Tables I and II).

These facts not only indicate that three types of mosaic may be spread in the field, but also confirm the theory previously advanced[19] that rugose mosaic may appear as partial infection of hills and tuber units as the result of uncontrolled field transmission occurring during the same season. Such current-season symptoms, as well as the incomplete or partial infection of the tuber, followed by a delayed manifestation of symptoms or by incomplete infection, often include spotting, streaking, and leaf-dropping (pl. 8, C and D).

TEST OF MOSAIC PERPETUATION THROUGH TRUE SEEDS

In 1921 the tubers and seed balls were saved together from each of a number of Green Mountain hills in a commercial field which contained about 5 per cent mild mosaic and which was infested with aphids. Some of the hills in question were mosaic and some were apparently healthy. The tubers were planted in the Orono, Me., greenhouse during the following winter and the seeds were planted in the same place in steam-sterilized soil. Seedlings were started in the winters of 1921–22 and 1922–23 and their first tubers were planted to produce a second generation. Of the original field-selected hills, hills 1 and 2 were mosaic in the field and produced, respectively, 2 and 3 mosaic tubers, and 7 healthy seedlings and no seedlings; hills 3 and 4 produced, respectively, 2 and 1 mosaic tubers, and 27 and 40 healthy seedlings; hill 5 produced 1 mosaic and 2 healthy tubers, and 6 healthy seedlings; hill 6 produced 2 healthy tubers and 32 healthy seedlings. Most of the seedlings in the second generation resembled Green Mountains closely, and were healthy in regard to the foliage. In brief, 1 mosaic plant (hill 1) produced 7 healthy seedling progeny while 3 apparently healthy plants (hills 3, 4, and 5) contracted mosaic which showed in their tuber-progeny but not in any of their 83 seedling-progeny.

EFFECT OF DIFFERENT CLIMATIC CONDITIONS IN 1922 ON MOSAIC LOTS FROM THE SAME TUBERS, AS SHOWN BY THE PROGENY GROWN IN NORTHEASTERN MAINE IN 1923

In order to ascertain the effect of climatic conditions on mosaic, seed pieces from the same tuber were planted in 1922 in three regions, namely, on Aroostook Farm in northeastern Maine, at Riverhead, Long Island, and at Nor-

[18] MURPHY, P. A. INVESTIGATIONS ON THE LEAF-ROLL AND MOSAIC DISEASES OF THE POTATO. Jour. Dept. Agr. and Tech. Instr. Ireland 23: 20–34, illus., 1923.
——— ON THE CAUSE OF ROLLING IN POTATO FOLIAGE; AND ON SOME FURTHER INSECT CARRIERS OF THE LEAF-ROLL DISEASE. Sci. Proc. Roy. Dublin Soc. (n. s.) 17: 163–184, illus., 1923.

[19] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 97

folk, Va. Thirty mild mosaic Bliss Triumph tubers and a similar Green Mountain lot were represented in each place. As previously reported,[20] observations were made in 1922 by the same person, one of the writers, in the three localities. The results of these observations indicated that mottling was less distinct on the foliage at Norfolk than in the other localities. It was also noted that most of the vines in the three localities showed mild mosaic symptoms; a few plants plainly disclosed mosaic-dwarf or bad mosaic symptoms. However, mosaic-dwarf appeared on the plants from the same tuber in each of the three localities and no tuber produced plants having mild mosaic in one locality and a different stage of mosaic in another locality. The tubers from each hill were kept separate and in 1923 were planted on Aroostook Farm where the results presented in Table X were obtained. Tuber decay in the Green Mountain series made comparison possible only in the Bliss Triumphs.

The data in Table X show that rugose mosaic was contracted by 12 of the hills in Virginia, by 7 in Long Island, and by 3 in Maine. Leaf-roll was contracted by 8 hills in Virginia and by 2 in Long Island. Spindle tuber was contracted by 21 hills in Virginia and by 4 in Long Island and in Maine. Mild mosaic probably was present in more hills in the Virginia lot than is apparent from Table X, because it would be masked, if present, in the hills with rugose mosaic.

EFFECT OF FOUR REGIONS ON THE DISSEMINATION OF MOSAIC, LEAF-ROLL, AND SPINDLE TUBER IN FIVE VARIETIES

In a previous publication [21] evidence was recorded indicating that the dissemination of insect-borne diseases both differed between localities and varied in the same locality, and that such lack of uniformity in dissemination apparently was due to differences and variations in the number, kind, and seasonal

TABLE X.—*Effect of different climatic conditions in 1922 on mosaic lots from the same tubers, as shown by the percentage of disease in the progeny grown in northeastern Maine in 1923*

Series No.[a]	Mild mosaic			Rugose mosaic			Leaf-rolling mosaic			Leaf-roll			Spindle tuber		
	Va.	L. I.	Me.	Va.	L. I.	Me.	Va.	L. I.	Me.	Va.	L. I.	Me.	Va.	L. I.	Me.
1	.....	100	100	50	12	.....	.....	.....	.....	50	75	.....	.....	.....	.....
2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100	100	100
3	33	100	100	55	.....	.....	.....	.....	.....	.....	.....	.....	100	50	.....
4	75	75	100	25	25	.....	.....	.....	.....	.....	.....	.....	75	.....	.....
5	.....	.....	100	.....	60	.....	.....	50	.....	100	.....	.....	100	.....	.....
6	40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	80	.....	.....
7	100	100	100	35	.....	.....	.....	.....	.....	.....	.....	.....	100	.....	.....
8	42	.....	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	100	.....	.....
9	.....	100	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	100	.....	22
10	.....	100	100	100	.....	.....	.....	.....	.....	.....	.....	.....	88	.....	.....
11	.....	.....	.....	75	50	.....	.....	.....	.....	100	.....	.....	(c)	.....	.....
12	.....	.....	.....	100	50	100	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	.....	100	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	100	.....	.....
14	.....	.....	100	100	.....	.....	.....	.....	.....	100	.....	.....	.....	100	.....
15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	100	75	.....
16	.....	100	100	.....	.....	.....	.....	.....	.....	44	.....	.....	100	.....	.....
17	.....	100	100	24	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	66
18	100	100	100	.....	11	.....	.....	.....	.....	.....	.....	.....	90	.....	.....
19	.....	100	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	100	.....	.....
20	.....	100	100	36	.....	.....	.....	.....	.....	100	.....	.....	100	.....	.....
21	.....	100	100	100	.....	.....	.....	.....	.....	.....	.....	.....	100	.....	.....
22	.....	.....	.....	.....	.....	.....	.....	100	100	18	.....	.....	.....	.....	.....
23	100	.....	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
24	18	100	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	81	.....	.....
25	100	100	100	.....	.....	.....	.....	.....	.....	32	.....	.....	.....	.....	.....
26	.....	100	.....	.....	.....	.....	45	.....	.....	.....	.....	.....	.....	.....	81
27	100	100	100	.....	.....	.....	.....	.....	.....	.....	.....	.....	50	.....	.....
28	.....	100	100	.....	16	.....	100	.....	.....	.....	.....	.....	100	.....	.....
29	.....	100	100	11	.....	9	33	.....	.....	.....	.....	.....	66	.....	.....
30	100	.....	.....	.....	.....	50	.....	.....	.....	.....	27	.....	.....	.....	.....

[a] Each series consisted of seed-pieces grown from the same tuber in 1922.
[b] Labels in series 1, 11–17 for Virginia were defaced, but sacks were taken in order of packing.
[c] Partly diseased.

[20] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 100.
[21] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 102, 109.

duration of certain insects known to be capable of the transmission of these diseases. In order to secure additional evidence regarding the effect of different regions on the dissemination of mosaic, leaf-roll, and spindle tuber, the following test was carried on.

In 1922 comparatively healthy commercial stocks of five varieties, Green Mountain, Rural New Yorker, Rose Four, Irish Cobbler, and Bliss Triumph, were grown in four regions: On Aroostook Farm in northeastern Maine, on Highmoor Farm in southwestern Maine, at Riverhead, Long Island, and at Norfolk, Va. In each variety one original stock was selected and divided into ten 30-pound lots. Two such lots of each variety were planted in each locality, one lot between rows of Green Mountains with 100 per cent both mosaic and spindle tuber and the other lot between rows of Irish Cobblers with 100 per cent leaf-roll and the same per cent spindle tuber. In addition, at Riverhead and Norfolk a third lot of each variety was grown several rods from any 100 per cent diseased stock. The diseased Green Mountains also were originally from one lot which was divided for planting in the four regions; the same is true of the diseased Irish Cobblers. In 1922 representative samples of the 50 different healthy lots and of the diseased lots were kept, and in 1923 were grown on Aroostook Farm, where the observations recorded in Table XI were made.

The data in Table XI can be used for several kinds of comparisons. Mild mosaic was present in about 20 to 30 per cent of the hills in the Green Mountain lots from between leaf-roll Irish Cobbler rows in the four regions, and from the isolated lots not grown between diseased rows. Relative to the preceding, there was a somewhat higher percentage in the Green Mountain lots from between mosaic Green Mountain rows in northeastern Maine and southwestern Maine, and still more from the same in Virginia and Long Island. Mild mosaic was present in about 30 to 40 per cent of all the Bliss Triumph lots except a very small, unrepresentative one where 3 of the 5 plants were leaf-roll. Mosaic, that is not of the rugose type, probably was present in the lots of the other varieties originally, and was unusually conspicuous in 1923 when present. Except where masked by rugose mosaic, it was present in about 30 to 50 per cent of the hills in the Rural New Yorker lots, in about 10 to 20 per cent of the Irish Cobbler lots, and in about 30 to 60 per cent of the Rose Four lots. Therefore, with these types of mosaic there was no marked difference between different regions or between different plots in the same region.

Rugose mosaic, however, was much more abundant in the Virginia lots than in the corresponding ones from other regions, in each variety and in all three locations in the field relative to diseased rows. The only exception is in a small lot of three plants, which is negligible. Rugose mosaic was present, though in small percentages, in a majority of the lots from northeastern Maine and Long Island but was absent from most of the lots from southwestern Maine. It was present in all Irish Cobbler lots except one, though this variety showed the lowest percentages, and absent from all Rural New Yorker lots except those from Virginia. Therefore rugose mosaic was contracted only slightly in southwestern Maine but markedly in Virginia, and was contracted by different varieties in different amounts.

Leaf-roll was in 100 per cent of every Virginia lot from between leaf-roll Irish Cobbler rows (pl. 10, A) and for the lots from Long Island ranged from 51 to 93 per cent (pl. 10, B), from southwestern Maine from 0 to 60 per cent (pl. 10, C), and from northeastern Maine from 0 to 22 per cent (pl. 10, D), with averages respectively of 74, 26, and 6 per cent. It is clear that the spread of leaf-roll was greater as the region was farther south. Farther spread than from diseased rows to adjoining healthy rows occurred in Virginia and Long Island but not in either part of Maine. In Virginia the Green Mountains and Bliss Triumphs between the mosaic Green Mountain rows contracted over 90 per cent leaf-roll, and where grown a few rods from 100 per cent diseased rows contracted over 80 per cent in that region. Where there was a difference among the varieties Green Mountains and Bliss Triumphs generally were the most infected by leaf-roll.

Spindle tuber was originally in 100 per cent of the hills in both the diseased Green Mountain lots, the diseased Irish Cobbler lots, and the "healthy" Rose Four lots. The last will be disregarded in the comparisons to be made. From between the Green Mountain rows the Virginia lots ranged from 68 to 92 per cent spindle tuber, the northeastern Maine lots from 30 to 90 per cent, the Long Island lots from 4 to 78 per cent, and the southwestern Maine lots from 4 to 38 per cent, with respective averages of 85, 54, 39, and 16 per cent. From between the Irish Cobbler rows the

TABLE XI.—*Effect of different regions on dissemination of mosiac, leaf-roll, and spindle tuber in different varieties*

Variety and region	Location in field, 1922	Disease in 1923					
		Total number hills July 22, 1923	Mosaic not of rugose type [a]	Rugose mosaic	Leaf-roll	Spindle tuber	Total disease percentages
Green Mountain:			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	213	31	1	1	45	78
S. W. Maine	do	149	32			7	39
Long Island	do	407	57	4	37	4	102
Virginia	do	[b] 377	41	22	99	92	254
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	188	20	1	2	32	55
S. W. Maine	do	110	16		47	7	70
Long Island	do	471	31	2	70	58	161
Virginia	do	[c] 289	25	20	100	98	243
Long Island	Not between 100 per cent diseased rows.	593	20	6	53	40	119
Virginia	do	[c] 240	23	42	81	78	224
Rural New Yorker:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	196	50			50	100
S. W. Maine	do	123	40			4	44
Long Island	do	341	46		11	50	107
Virginia	do	220	27	22	37	88	174
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	143	29		2	34	65
S. W. Maine	do	103	33			18	51
Long Island	do	264	25		67	48	140
Virginia	do	[c] 28	4	73	100	85	262
Long Island	Not between 100 per cent diseased rows.	237	46		36	24	106
Virginia	do	68	19	13	20	36	88
Irish Cobbler:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	218	15	2		90	107
S. W. Maine	do	77	14	4		38	56
Long Island	do	242	7	2	38	78	125
Virginia	do	631	12	12	16	92	132
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	203	12	3	2	96	113
S. W. Maine	do	41	17	2	19	34	72
Long Island	do	214	17		89	76	182
Virginia	do	[c] 80	5	11	100	100	216
Long Island	Not between 100 per cent diseased rows.	192	20	1	17	74	112
Virginia	do	621	11	22	15	72	120
Bliss Triumph:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	204	35			30	65
S. W. Maine	do	6	33			16	49
Long Island	do	263	43	4	74	22	143
Virginia	do	[c d] 212	24	45	93	68	230
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	251	32			6	38
S. W. Maine	do	5			60		60
Long Island	do	255	29	4	93	8	134
Virginia	do	[c] 205	31	33	100	32	196
Long Island	Not between 100 per cent diseased rows.	233	40	2	38	6	86
Virginia	do	[c] 148	32	22	84	18	156
Rose Four:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	158	22	5		(e)	27
S. W. Maine	do	94	48			(e)	48
Long Island	do	256	38	9	6	(e)	53
Virginia	do	[c] 124	19	25	30	(e)	74
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	112	54	1	22	(e)	77
S. W. Maine	do	50	52		6	(e)	58
Long Island	do	262	38	10	51	(e)	99
Virginia	do	[d 3]	33		100	(e)	133
Long Island	Not between 100 per cent diseased rows.	283	58	1	7	100	166
Virginia	do	[c] 67	31	10	37	100	178

[a] Mild mosaic in the Green Mountains and probably in the Bliss Triumphs, and of undetermined type in the other varieties.
[b] All small except three healthy hills.
[c] All small.
[d] Many missing hills.
[e] Not examined because completely diseased in 1922.

Virginia lots ranged from 32 to 100 per cent, the Long Island lots from 8 to 76 per cent, the northeastern Maine lots from 6 to 96 per cent, and the southwestern Maine lots from 0 to 34 per cent, averaging, respectively, 79, 48, 42, and 15 per cent. Evidently spindle tuber increased more in Virginia than elsewhere, and less in southwestern Maine. A comparison of varieties as to susceptibility can not be made because the Green Mountains were the only one of the "healthy" stocks to be originally free from spindle tuber.

The total disease percentages are higher for Virginia in every variety whether from between diseased Green Mountain rows or from between diseased Irish Cobbler rows. For all varieties the total for Virginia is about double that of northeastern Maine, about triple that of southwestern Maine, and about half again greater than for Long Island. The total for Virginia probably should be larger because of mild mosaic being masked by rugose mosaic. It may be pointed out here that the test in 1922 in northeastern Maine was carried out in a field containing many plots with high percentages of disease of various types; in southwestern Maine in an isolated plot with the climatic conditions unusually unfavorable for growth; and on Long Island and in Virginia in plots surrounded by commercial varieties.

A few definite conclusions can be drawn from the preceding comparisons. Proximity to mild mosaic rows in 1922 helped to increase the amount of mild mosaic contracted by Green Mountains, more in Virginia and on Long Island than in Maine. Rugose mosaic was contracted markedly in Virginia but not elsewhere. While the "healthy" stocks, exclusive of the Green Mountains, were originally more or less infected with the milder types of mosaic and with spindle tuber, they were free from leaf-roll. The spread of this disease was greater farther south, and in Virginia and on Long Island was not restricted to spread from leaf-roll rows to adjoining rows. Spindle tuber increased more in Virginia than elsewhere. The total disease percentage increased most in Virginia and least in Maine.

One practical application of these conclusions is that the frequent statement to the effect that a hot climate causes rapid degeneration of potatoes, need not assume a direct effect, since an indirect effect by increasing the spread of degeneration diseases is now known to exist. It is also now more explicable why commercial stocks in northeastern Maine that are found to contain considerable leaf-roll usually can be traced back to a more southern source. It is plainly probable that the mosaic familiar to southern buyers of potato seed is not of the same type as that most often found in Maine. Emphasis is here given to the claim previously made [22] that the degeneration problem "because of its complexity may vary greatly from one locality to another" so that "control measures must be worked out for different sets of conditions, following research based initially on the general principles now fairly well understood."

A satisfactory explanation of these results would require more detailed study than has been made of each region relative to the presence of the various degeneration diseases in weeds and in neighboring commercial stocks, to the kinds, numbers, and development of infestations of transmitting insects, and to the effects of the diseases upon the development and maturing of the different varieties. Improvement should be made upon the methods used in making this test, especially in regard to original freedom from disease except for the one or two desired in any diseased stock. The requisite control measures to accomplish this are not yet fully understood.

SUMMARY

(1) Previously reported results with at least seven distinct degeneration diseases of potatoes suggest several new problems. A review of the symptoms includes several new ones, namely, spindling sprouts as an occasional symptom of leaf-roll even when unaccompanied by net necrosis, streaks and spots on corollas as symptoms of streak, and tuber cracking as a current-season symptom of unmottled curly dwarf inoculation. A fourth type of mosaic, "crinkle mosaic," is also distinguished tentatively; this is unrelated to "crinkle."

(2) Leaf-mutilation inoculations made in the field within the Green Mountain variety caused infection with mild mosaic, leaf-rolling mosaic, rugose mosaic, spindle tuber, unmottled curly dwarf, and streak, and also with various combinations of these diseases. This was followed by their natural spread from inoculated hills to adjacent hills, especially by mild mosaic.

[22] SCHULTZ, E. S., and FOLSOM, D. Op. cit., p.112.

(3) Intervarietal leaf-mutilation inoculations of Green Mountains transmitted mild mosaic and crinkle mosaic from Bliss Triumphs; leaf-rolling mosaic, rugose mosaic, spindle tuber, and unmottled curly dwarf from Rurals; rugose mosaic from seedling lots; and leaf-rolling mosaic, rugose mosaic, and spindle tuber from Irish Cobblers. Varietal modification of symptoms was disclosed, mottling especially being suppressed by Rurals and Irish Cobblers.

(4) Intervarietal leaf-mutilation inoculations in insect cages showed that a repetition of the inoculation favors infection with mild mosaic and spindle tuber; that every one of several varieties was susceptible to spindle tuber; that Irish Cobblers either are symptomless carriers of mild mosaic or are resistant or immune to this disease; that "mosaic dwarf" sometimes is a combination of rugose mosaic, spindle tuber, and streak; that there is a varietal difference in susceptibility to streak; and that with conditions apparently similar not only will results of inoculations sometimes be negative where experience would lead one to expect infection, but there even may be a separation of viruses originally in combination in a given inoculum.

(5) Aphids sometimes do not transmit disease under conditions that apparently are the same as those giving positive results.

(6) With regard to current-season symptoms, a progressively smaller amount of infection was induced by leaf-mutilation inoculation with the leaflets, entire shoots, stems and petioles together, seed tubers, and roots, respectively, of rugose mosaic plants. Similar inoculation with juice from mild mosaic Green Mountain shoots and from certain mottled Irish Cobbler shoots, and the use of the latter in grafts, produced no apparent effects. Inoculation with the seed-cutting knife gave negative results with seven diseases and several combinations.

(7) Leaf mutilation inoculations in insect cages produced current-season symptoms in Green Mountains with progressively shorter incubation periods for mild mosaic, leaf-rolling mosaic, and rugose mosaic, but only rugose mosaic induced current-season symptoms in Rural New Yorkers and Irish Cobblers. Since these varieties are susceptible to leaf-rolling mosaic, this shows for this disease either a longer incubation period or a lower degree of varietal susceptibility correlated with varietal suppression of mottling.

(8) Aphid inoculations in insect cages produced current-season symptoms in Green Mountains with four types of mosaic—mild, leaf-rolling, rugose, and crinkle mosaic. Here the current-season symptoms of rugose mosaic following aphid inoculation were less marked than after parallel leaf-mutilation inoculations, which was also true in Rural New Yorkers and Irish Cobblers. Mild mosaic in at least the eighth consecutive generation of a Green Mountain strain was still mild and was distinct from rugose mosaic and the other types, contrary to prevailing theories of progressive increase in severity that apparently are based on general field observations made where the more severe types can increase. Facilitation of experimentation is obtained from the use of insect cages with openings in the top.

(9) Other aphid inoculations produced, in addition, current-season symptoms of three disease combinations—rugose mosaic and spindle tuber, crinkle mosaic and spindle tuber, and leaf-rolling mosaic and leaf-roll.

(10) Root and foliage contact with spindle tuber plants under insect-free greenhouse conditions resulted in no transmission. In another greenhouse, contact of roots and leaves, and of leaves in the absence of aphids and leaf mutilation but in the presence of other kinds of insects, was accompanied by the dissemination of mosaic but not of leaf-roll.

(11) A third species of aphids, the "buckthorn aphid" (*Aphis abbreviata* Patch), has been found to be common on potatoes and to be capable of transmitting mild mosaic, at least. Its early appearance has been accompanied by

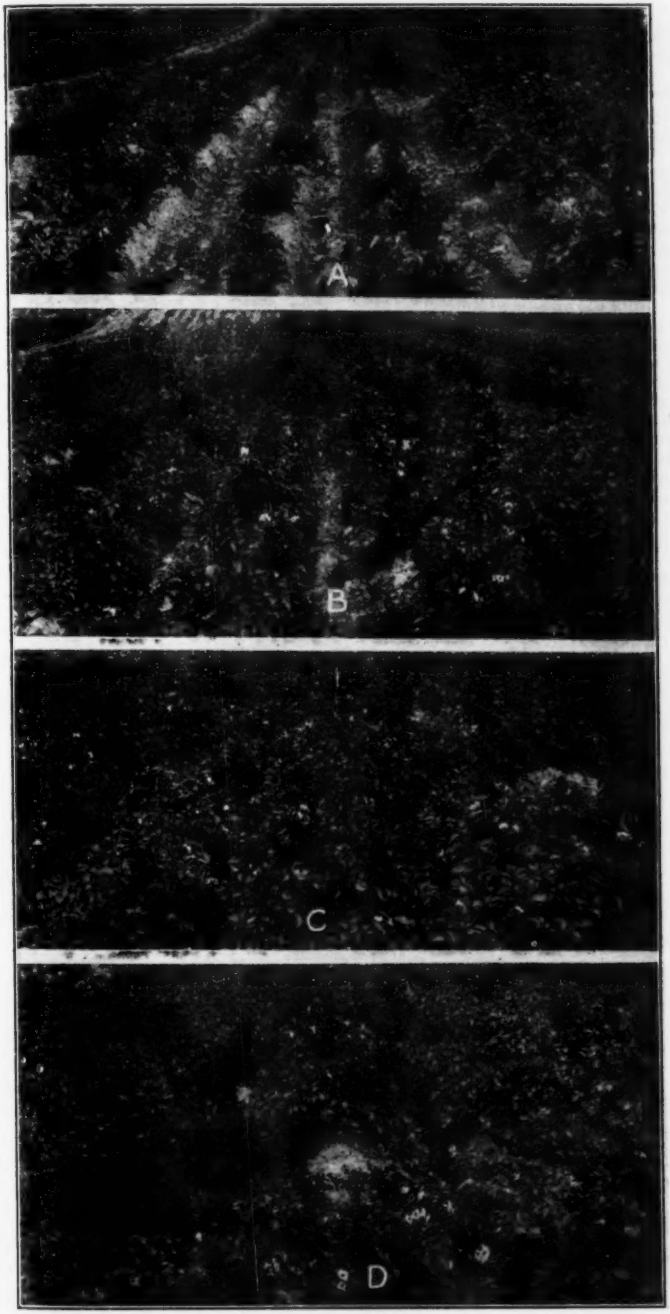
EXPLANATORY LEGEND FOR PLATE 10

A.—The two middle rows are progeny of Green Mountains planted in Virginia between leaf-roll rows (left) and between mosaic rows (right). Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, B, C, and D

B.—The two middle rows are progeny of Green Mountains planted on Long Island between leaf-roll rows (right) and somewhat isolated (left). Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, A, C, and D

C.—The middle row is progeny of Green Mountains planted on Highmoor Farm in southwestern Maine between leaf-roll rows (in foreground) and between mosaic rows (beyond stake). Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, A, B, and D

D.—The middle row beyond the gap in the foreground is progeny of Green Mountains planted on Aroostook Farm between leaf-roll rows. Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, A and C



(For explanatory legend see p. 526)

the natural spread of rugose mosaic and leaf-rolling mosaic with the appearance of current-season symptoms. These include spotting, streaking, and leaf dropping, which also may result from delayed symptoms or incomplete infection which sometimes follow tuber perpetuation.

(12) Ninety healthy seedlings were grown from seeds produced by several Green Mountain plants, although the parent plants either were affected with mosaic or had mosaic tuber progeny.

(13) Mild mosaic Bliss Triumph sister hills (from the same seed tubers) planted in three regions showed less distinct mottling in Virginia but contracted more rugose mosaic, leaf-roll, and spindle tuber there than on Long Island and contracted least of all in northeastern Maine, as shown by the progeny all grown in northeastern Maine.

(14) Comparatively healthy commercial stocks of five varieties divided and grown in four regions mostly next to diseased rows, with the progeny all grown in one place, showed that mild mosaic spread more in Virginia and on Long Island than in southwestern and northeastern Maine; that rugose mosaic was contracted markedly in Virginia but not elsewhere; that leaf-roll spread more farther south; that spindle tuber increased more in Virginia than elsewhere; and that the total disease percentage increased most in Virginia and least in Maine. It is therefore concluded that a hot climate may cause degeneration of potatoes indirectly through favoring the spread of diseases. It also suggests why northern-grown seed is preferred; how buyers and sellers of seed if from different regions may misunderstand each other in discussing "mosaic," because of familiarity with different types; and why the potato degeneration problem requires local or regional study.

STUDIES OF SPORE DISSEMINATION OF VENTURIA INAEQUALIS (CKE.) WINT. IN RELATION TO SEASONAL DEVELOPMENT OF APPLE SCAB [1]

By C. N. FREY,[2] *formerly Assistant in Plant Pathology, University of Wisconsin,* and G. W. KEITT, *Professor of Plant Pathology, University of Wisconsin*

During the season of 1916 it became apparent to the junior author, who was then beginning work on the control of apple scab,[3] that further studies of certain details of the life history of the causal fungus in relation to the seasonal development of the disease under local conditions were fundamental to the satisfactory application of control measures. A clearer understanding of certain aspects of spore dissemination and infection appeared to be of primary importance. Accordingly, in the spring and summer of 1917, some studies of these questions were undertaken as a minor problem in such time as the writers could devote to it. The advent of the war prevented continuation of this work, and subsequently pressure of duties in another field upon the senior author has prevented earlier publication of the results. However, the junior author and his coworkers have extended these investigations and plan to supplement the following account in a later paper.

A STUDY OF THE SPORE CONTENT OF ORCHARD AIR

METHODS

Of the various methods for the study of spore dissemination described in the literature with which the writers are conversant, none appeared exactly to meet the requirements of their problem. Consequently an attempt was made to devise one.

ELECTRICAL PRECIPITATION

At the suggestion of L. F. Hawley of the Forest Products Laboratory of the United States Department of Agriculture, an attempt was made to adapt to the purposes of the present study the electrical method described by Cottrell [4] for removing suspended particles from gases. Preliminary experiments showed that when spores of *Venturia inaequalis*, *Ustilago zeae*, or certain other organisms were brought within the field of a static machine most of them became negatively charged and passed to the positive pole. A few of the spores tested became positively charged, a small number of each kind always passing to the negative pole.

These observations encouraged the writers to pursue their experiments somewhat further. A glass tube 12 inches long and 1.5 inches in diameter was set up between the poles of the static machine. In each end of this tube was placed a rubber stopper through which ran two glass tubes, each 2 inches long and one-fourth inch in diameter. One of these tubes was attached to a vacuum pump, while the opposite one was left open to permit the access of air. A copper wire was passed through the other two tubes in such manner as to run through the middle of the larger tube and to attach at one end to the negative pole of the static machine. All openings into the larger tube were sealed with wax, with the exception of the two small tubes to permit the ingress and egress of air. Tin foil was lightly wrapped around the outside of the large tube and connected with its inner wall. A copper wire attached to the foil was connected with the positive pole of the static machine. A thin coat of vaseline had previously been rubbed on the inside of the tube to aid in holding the spores as they were precipitated against it. Air currents bearing abundant fungus spores were then passed through the tube while the static machine was in operation. When the current of air was suitably regulated and the static machine delivered a high potential, about 30,000 to 50,000 volts, most of the spores were caught on the first 3 or 4

[1] Received for publication May 24, 1924; issued June, 1925. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

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[3] A comprehensive account of this disease and its causal fungus, *Venturia inaequalis* (Cke.) Wint., with an extensive bibliography, is given in the following citation: WALLACE, E. SCAB DISEASE OF APPLES. N. Y. Cornell Agr. Exp. Sta. Bul. 335, p. 545–624, illus. 1913.

[4] COTTRELL, F. G. THE ELECTRICAL PRECIPITATION OF SUSPENDED PARTICLES. Jour. Indus. and Engin. Chem. 3 : 542–550, illus. 1911.

inches of the tube, being deposited in a thin film on its inner wall. When smut spores were used the tube was quickly coated with a black layer, the darkest zone being situated near the inlet, showing that they were precipitated very promptly upon entering the charged field. When the apparatus was suitably adjusted very few spores passed through the tube. This was determined by passing the outgoing air through a wash bottle containing oil and water and examining the fluid microscopically. Only occasional spores were found.

Preliminary steps were then taken toward developing an electrical precipitation apparatus which might be operated satisfactorily under orchard conditions. However, pressure of work, expense of the electrical equipment necessary, and the fact that very satisfactory results were being obtained by the method described below led to the discontinuance of experiments with the electrical method. The writers believe, however, that this method has possibilities of adaptation to problems involving a study of the spore-content of air. The results obtained appear to be in general agreement with those of Buller (p. 192–195) [5], who has studied the electrical charges on spores from a different point of view.

FILTRATION ON MEMBRANES [6]

Early in the consideration of the problem it occurred to the writers that it might be possible to devise an apparatus which might be run continuously and so constructed that spores could be filtered from the air upon transparent or translucent membranes on which they might be counted under the microscope. Preliminary tests with membranes prepared from cloth, fiber, gelatin, nitrated cellulose, cellulose acetate, and viscose were unsuccessful. The three latter-named materials were not sufficiently porous to allow adequate passage of air. Pure filter paper was tried, and it was found that, by specially treating [7] soft Swedish filter paper, nearly pure cellulose, there was produced a tough, waterproof membrane which proved to be satisfactory. Some special Swedish filter papers and Whatman's hardened filter paper were then investigated and found to be sufficiently waterproof to resist disintegration when wet by rain. The paper, however, should not be so altered by treatment with acid that it loses its porosity and precludes adequate passage of air.

A motor-driven vacuum pump was then set up and a heavy-walled rubber tube was used to connect its intake with a special apparatus for holding the filter paper (fig. 1). It consisted of a thick-walled brass tube (A) on the machined end (B) of which was screwed a very closely fitting brass cap (C) in the middle of which was an aperture 1 inch in diameter. A disc (D) of filter paper of suitable diameter, underlain by a supporting disk of fine silk fabric of similar size, was placed upon the machined end of the metal tube, and the cap screwed tightly over it. In this way it was held firmly in position, the cap making a joint that was air-tight, while a circular area 1 inch in diameter was exposed for purposes of filtration. A perforated rubber stopper (E) connected the device with the suction tube by means of a glass tube. Numerous tests of the capacity of the machine with different filters of the type used throughout the experiments showed that approximately 1,200 liters of air passed through the apparatus in an hour.

The membranes were removed at intervals as circumstances warranted. As soon as a membrane was removed it was placed in a small Esmarck dish and treated with a small amount of glycerine to make sure that the spores remained in place. In each test a membrane similar to the one used for filtration was placed beside the latter, collected with it, and studied microscopically, as a control against chance accumulation of air-borne ascospores. Studies of these, however, showed so few spores that it seemed unnecessary to make any correction in the results for them. The number of spores caught on the membrane was determined by direct counts under the microscope. The olivaceous color and characteristic form and size of the spores made them easily observed without staining. If the number was

[5] BULLER, A. H. R. RESEARCHES ON FUNGI. v. 1, illus. London, New York, etc. 1909.

[6] In later studies the junior author and his coworkers have developed another filtration technique which is being used in their further work. Orchard air is drawn by means of a motor-driven suction apparatus through a suitably arranged filter of nitrocellulose. Thence it passes through a gas meter which records the volume. At the end of the run, the nitrocellulose filter, bearing the spores caught, is dissolved in a suitable glass container in a mixture of alcohol and ether and allowed to evaporate to a gel. The ascospores settle to the bottom and their number is computed on the basis of microscopic counts. This is an adaptation of a technique developed by Pasteur. It will be described in more detail in a later paper.

[7] The filter paper was dipped for about one-half minute in nitric acid, sp. gr. 1.42, transferred for a brief period to sulphuric acid (equal volumes of 1.84 sp. gr. sulphuric acid and water), and washed in water and then in a weak solution of ammonia.

small or nil the entire surface of the exposed area of the membrane was observed. This was accomplished and duplication of observation avoided by the use of a mechanical stage and a micrometer. If spores were numerous, counts were made of at least 30 fields, chosen at random but without duplication from all parts of the filter, and the total number was computed on the basis of this average. The results thus obtained agreed closely with those from counts over the entire exposed area. A Leitz microscope was used, with a No. 4 eyepiece, a No. 6 objective, and a tube length of 170 mm.

FIELD EXPERIMENTS

During late April and early May preliminary observations were made to determine the time at which ascospores would be sufficiently mature for natural discharge. Over-wintered apple leaves bearing numerous perithecia of *Venturia inaequalis* were brought into the laboratory daily, thoroughly moistened, and placed in moist chambers in such position that one could ascertain by miscroscopic examination of the glass surface below them whether or not ascospores had been discharged. No discharge from such material was observed until May 7.

On May 6, just as the cluster buds of the apple were beginning to open, the apparatus was installed in the Turville[8] orchard, about 1½ miles south of Madison. This orchard, consisting of about 50 large trees planted in four rows running east and west, was in sod, and the ground under and about the trees was abundantly littered with over-wintered leaves which bore perithecia of *Venturia inaequalis* in great profusion. The apparatus was placed under the outer branches of a Fameuse tree which stood in the southernmost row, second from the eastern end. The membrane was placed at elevations varying from 1½ to 3 feet above the ground, and was protected from rain by a small roof-like shelter which was raised sufficiently to permit free passage of air over the filter. The machine was run continuously, except for brief stops for changing membranes and caring for the motor, until June 19, and subsequently at intervals. The results are summarized in Table I in correlation with hourly records of rainfall. Hourly records of temperature and wind velocity show valuable correlations but are not included in the table because of limitations of space.

The field and laboratory observations indicate that on May 6, when the field filtration tests were started, the ascospores were just approaching maturity. Apparently no discharge had occurred prior to this time. During the dry period from May 6 to 18 no Venturia spores were caught. On May 19, rain fell from about 2 to 5

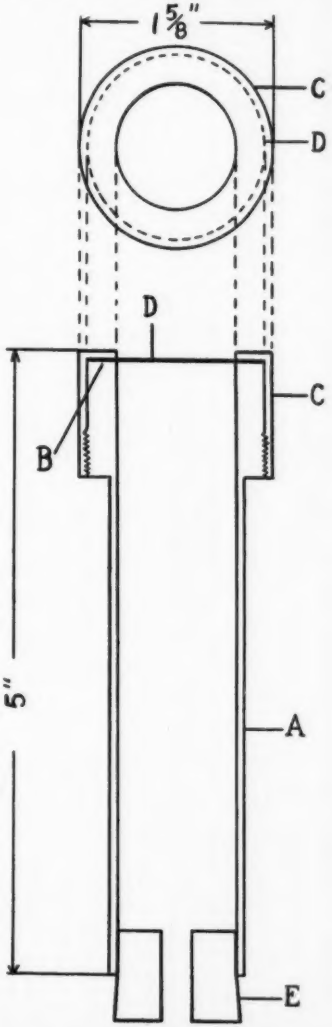
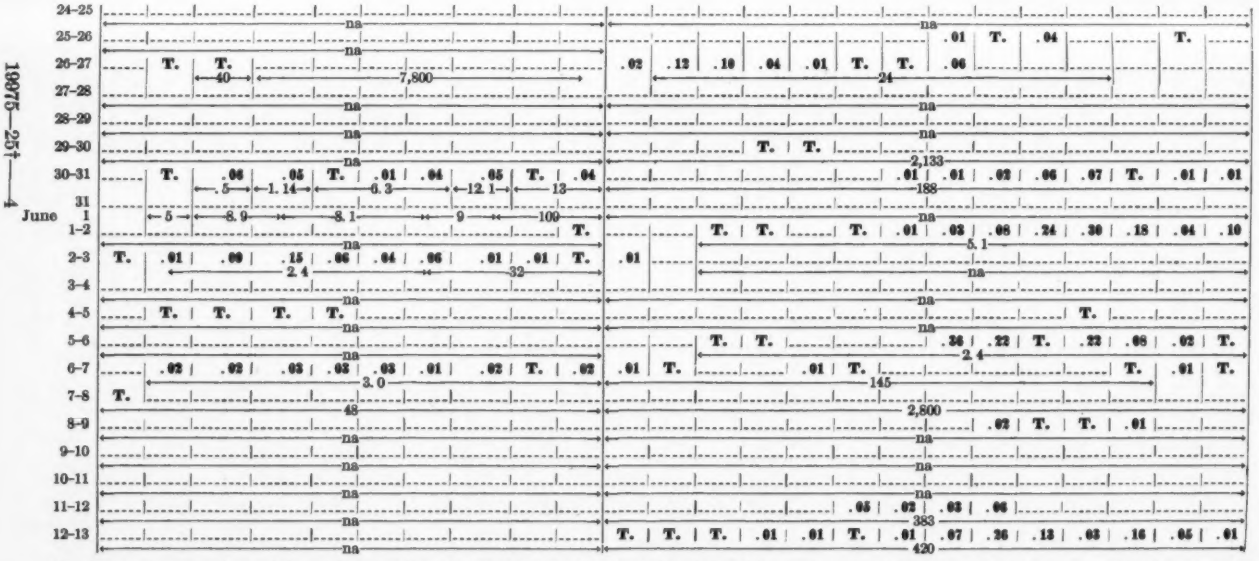


FIG. 1.—Device used for filtering spores from the air

[8] Grateful acknowledgments are made to Thomas and Wm. D. Turville for their kindness in making their orchard available for this work.

TABLE I.—*Results of filtration experiments, showing orchard air content of ascospores of V. inaequalis in relation to hourly records of rainfall, Madison, Wis., 1917*

Dates	Records by hours a																						Total rainfall (inches)
	A. M.				P. M.												A. M.							
	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
May 5–6																									
6–7	na										na														0
7–8	na										na														0
8–9	na										na						T.					T.			T.
9–10	na										na														0
10–11	na										na														0
11–12	na										na														0
12–13	na										na														0
13–14	na										na														0
14–15	na										na														0
15–16	na										na														0
16–17	na										na														0
17–18	na										na														7
18–19	na										na														0
19–20	na																T.	.54	.23						.77
20–21	89				204												840					T.			T.
21–22	.01	.09	.23	.17	.16	.03	T.	T.						T.	.11	T.	T.	T.	.08	.02	.01	.03	.05	.03	.33
22–23	.07	.04	.05	.03	.01												(?) b	.01	.05	T.	.03	.05	.05	.07	.07	.04	1.06
23–24																	23								.20
24–25																	100								0
	na																na								



[a] In the space representing each hour, the upper line (the line of the date) is devoted to the rainfall record, expressed in inches. In order to make the rain periods stand out more sharply, zeros are omitted and bold-faced type is used. "T" indicates that only traces of rain fell.

The lower line gives the ascospore discharge record. The arrows indicate the periods during which the filter was operated. The numbers between the arrows represent the average number of liters of air filtered for each ascospore caught. *"na" indicates that no ascospores were caught during the period shown*, though air was filtered at the standard rate of approximately 1,200 liters per hour.

The data on rainfall are taken from the records of the Madison station of the U. S. Weather Bureau, which is situated on the top of a four-story building about 1½ miles from the Turville orchard. These data are therefore not to be interpreted as representing more than a close approximation of the conditions which obtained in the orchard. Inches of rainfall on the dates omitted from the table follow: June 20–21, 0.03; 22–23, 1.24; 23–24, 0.01; 24–25, 0.01; 25–26, 0.53; 27–28, 0.45; 28–29, 0.60; 29–30, 0.10; July 5–6, 0.03; 6–7, 0.07; 10–11, 0.03; 13–14, 0.15.

[b] The filter was so covered by sleet that no reliable record could be obtained.

19675—25†——4

TABLE I.—*Results of filtration experiments, showing orchard air content of ascospores of V. inaequalis in relation to hourly records of rainfall, Madison, Wis., 1917*—Continued

Dates	Records by hours																								Total rainfall (inches)
	A. M.				P. M.												A. M.								
	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
June 13–14	T.	T.	.02								T.	.01													.03
14–15			T.	T.	T.			T.	T.	T.	T.	T.				.01	137								.01
15–16											1,000														0
16–17	na										na			T.	T.		na								T.
17–18	na										na						na								0
18–19	na										na						na								0
19–20	na										na						na								0
July 30	na										na						na								0
1	na										.01	.08					.51	.02							62
1–2	na										30														0
2–3	na										na						na								0
3–4	na										na						na								0
11–12		.14			T.		T.			T.	.01	T.					na								0
12–13	na										33														.15
17–18								.07	.01			.02	T.				56								0
18–19											.07	T.					na								.10
19–20											na						na								.07
21–22	na										na						.08	1.28							0
											na														1.36

a. m., and the first discharge of the season was recorded by the apparatus. Ascospores were caught at the average rate of 1 ascospore to 30 liters for the 3-hour period covered. From 8.30 to 10.30 a. m., while the leaves were still wet, the average rate of catch was 1 ascospore to 89 liters. From 10.30 a. m. to 6 p. m. it was 1 to 204 liters; and from 6 p. m., May 19, to 8 a. m., May 20, 1 to 840 liters. No spores were caught from 8 a. m. to 6 p. m., May 20, by which time the leaves had thoroughly dried.

During the night of May 20–21 rain or sleet fell almost continuously and rather high winds prevailed. Unfortunately, in spite of the shelter which had been erected to protect the membrane from weather, it was so washed by rain and sleet that no trustworthy record could be taken. In the morning, however, the winds became less violent. The rain continued from 9 a. m., when a new membrane was put on, until about 4 p. m. From 9 a. m. to 5.30 p. m. ascospores were caught at the average rate of 1 ascospore to 0.4 liters. This proved to be the heaviest discharge recorded during the experiments. The wind velocity for the period of this run varied from 14 to 23 miles an hour.

Rain began to fall about 11 p. m. May 21 and continued until about 1 p. m. May 22. From 7 p. m., May 21, to 7 a. m., May 22, ascospores were caught at the average rate of 1 ascospore to 23 liters. The wind velocity for the period during which this rain fell varied from 21 to 34 miles an hour (NE. to NW.). No record is available for the daylight hours of May 22. The machine ran from 6 p. m., May 22, to 8 a. m., May 23, and recorded an average catch of 1 ascospore to 109 liters. Apparently the leaves were sufficiently moist from the morning's rain to continue this light discharge. Following thorough drying the next morning, discharge ceased and no spores were caught until the next rain.

Light showers fell between 1 and 7 a. m., May 26, but no record for this period is available. From 10 to 11 a. m., however, during traces of rainfall, the machine was run, and ascospores were caught at the rate of 1 ascospore to 40 liters. From 11 a. m. to 5.30 p. m., with no additional rainfall, the catch was only 1 to 7,800 liters.

Showers fell during the night of May 26–27, and spores were caught at the rate of 1 ascospore to 24 liters. The wind velocity during this rain period varied from 10 to 22 miles.

No more ascospores were registered by the machine until the night of May 29–30, when, following traces of rain, they were caught at the rate of 1 ascospore to 2,133 liters. On May 30, however, an excellent opportunity for a fractional study of a discharge period presented itself. From 9 to 10 a. m. a trace of rain fell, followed by a fairly heavy rain from 10 to 12, a trace from 12 to 1, and almost continuous rain throughout the remainder of the afternoon. The wind velocity from 10 a. m. to 6 p. m. varied from 13 to 23 miles an hour. From 10 to 11 a. m. ascopores were caught at the average rate of 1 ascospore to 0.5 liters; from 11 to 12, 1 to 1.14; from 12 to 3, 1 to 6.3; from 3 to 4, 1 to 12.1; from 4 to 6, 1 to 13. From midnight until 8 a. m., May 31, it rained almost continuously. However, from 6 p. m., May 30, to 8 a. m., May 31, the average rate of catch was only 1 ascospore to 188 liters. The old leaves on the ground remained wet in the tall grass of the orchard until about 3:30 p. m., May 31. By this time the wind, which had freshened about 3 p. m., had begun to dry them perceptibly. This is very well reflected in the ascospore content of the air. From 9 to 10 a. m., May 31, spores were caught at the rate of 1 ascospore to 5 liters of air; from 10 to 11.30, 1 to 8.9; from 11.30 to 2.30, 1 to 8.1; from 2.30 to 3.40, 1 to 9; from 3.40 to 6, 1 to 109. It appears that, at the beginning of this rain period, there was an abundance of mature ascospores ready for discharge. During the first 2 hours (10 to 12 a. m., May 30) the rate of discharge was very high. Subsequently, however, it dwindled rapidly, and relatively few mature spores appear to have been available for discharge during the rain of the following night. The rate of discharge increased very markedly on the morning of May 31, probably due to the maturing of asci during the current moist period.

No further discharge was recorded until the rain of the night of June 1–2, when ascospores were caught at the rate of 1 ascospore to 5.1 liters (8 p. m. to 8 a. m.). From 9.30 a. m. to 2.30 p. m., June 2, it rained continuously, and ascospores were caught at the rate of 1 ascospore to 2.4 liters. From 2.30 to 6 p. m., although showers continued, the rate of catch fell to 1 to 32 liters. During the following night, no spores were caught. In this instance, the period of rapid discharge was considerably more prolonged than was the case in the preceding rain.

No further discharge was recorded until the rain of the night, June 5–6, when the average rate of catch from 8 p. m. to 8 a. m. was 1 ascospore to 2.4 liters. The rain continued through-

out the day of June 6, the catch from 9 a. m. to 6 p. m. being at the rate of 1 to 3 liters. During the following night, although there were slight showers, the rate fell to 1 ascospore to 145 liters. In this, as in the preceding instance, the period of rapid discharge was fairly prolonged, probably extending over about 15 hours. It appears likely that the more extended periods of these later heavy discharges, as compared with that of May 31, are to be attributed to a more generally advanced stage of maturity of asci and possibly to more rapid ripening.

Minor discharges occurred during the showers of June 7, 12, 13, 14, and 15. Although fairly heavy rains fell on June 12 and 13, the discharges were very light. It appears that the heavy discharges on and prior to June 7 went far toward exhausting the supply of ascospores. The failure to catch any spores whatever during the rainfall of 0.03 inch of June 9 is not understood. It is possible that there was less precipitation in the orchard than at the weather station. It is conceivable, on the other hand, that the supply of ripening asci was so nearly exhausted that no significant numbers were in condition to discharge at this time.

No spores were caught during the dry period from June 16 to 20.

Due to the absence of the senior author, no record was taken for the period of June 21 to 29.

Rain fell in the latter part of the night of June 30–July 1. From 6 p. m. to 8 a. m., the average rate of catch was 1 ascospore to 30 liters. When it is considered that most of the discharge of this period evidently occurred from about midnight to 5 or 6 a. m., it is apparent that this represents a fairly heavy discharge for so late a date.

During the dry period, July 2 to 4, no ascospores were caught.

The next run was made on the night July 11–12, when light showers fell from about 6 to 8 p. m. The catch was at the rate of 1 ascospore to 33 liters. From 8.30 a. m. to 6 p. m. July 12, there was no rain, and no spores were caught.

The next run was made on the night of July 17–18. Light showers fell between 7 and 9 p. m. From 6 p. m. to 8 a. m. the rate of catch was 1 ascospore to 56 liters.

Showers fell from 6 to 8 p. m., July 18. No ascospores were caught in a run from 6 p. m., July 18, to 8 a. m., July 19. During the following 24 hours, no rain fell and no spores were caught.

From 2 to 4 a. m., July 22, 1.36 inches of rain fell. From 6 p. m., July 21, to 8 a. m., July 22, no ascospores were caught. It appears that the season's supply was exhausted at this time.

From the results of these experiments it is readily apparent that the presence of an adequate amount of water is a primary requisite for the ejection of ascospores of *Venturia inaequalis*. Under the conditions of the tests, dew alone was not sufficient to occasion discharges of any considerable consequence. It is apparent, further, that rain water is very important, not only in relation to the discharge of ascospores, but for their maturation as well. This latter point has been more clearly established in later studies by the junior author and his coworkers and will be discussed in a later paper.

The records of May 31, June 3, and June 6–7 show that when ascospores in abundance are in condition to be ejected, heavy discharges occur at the beginning of a rain period and continue during continuous rain as long as the supply of ripe asci lasts. The periods of heavy discharge varied from several hours on May 31 to about 15 hours on June 3 and June 6–7.

The maximal average concentration of spores for a single run of the machine, 1 ascospore to 0.4 liters, was obtained between 9 a. m. and 5.30 p. m., May 21. When one considers that only traces of rain fell after 2 p. m., it appears likely that, at the period of maximal discharge, the concentration of spores may have been considerably greater than the average for the entire run. In order to gain a still more concrete conception of such concentrations of spores it seems to be of value to compute on the basis of the records just mentioned the number of ascospores which would have passed in the 8½-hour period studied through an imaginary orifice 10 cm. square interposed 3 feet above ground perpendicular to the wind direction. With the average wind velocity for this period of 18.5 miles an hour and the average ascospore content of the air 3 feet above ground of 1 ascospore to 0.4 liters, as shown by the records, it appears that over 6,300,000 ascospores would have passed through the orifice postulated.

In considering these records of ascospore discharge in relation to the seasonal development of the host plant and to control measures it would appear that they are in accord with the widely prevalent idea that the first ascospore discharges of *Venturia*

inaequalis are to be expected at about the time the fruit buds separate in the clusters just prior to the blooming period. Subsequent studies, however, have shown that, in the vicinities of Madison and Sturgeon Bay, Wis., ascospore discharge ordinarily begins at a much earlier stage of bud development and that the lateness of the initial discharge of 1917 may be attributed largely to the paucity of rainfall in early April and the first 18 days of May. It should be noted that even in 1917 it was shown by wetting leaves freshly collected from the orchard that asci were capable of discharging spores as early as May 7.

Although the air filtration experiments were planned primarily for the study of ascospore dissemination a careful watch was kept for conidia. After June 2, when the first scab lesions were observed, conidia began to appear in small numbers on the membrane in rainy periods, particularly when the rain was accompanied by high winds. They were never caught, however, except under these conditions. These facts led the writers to make certain observations upon the conditions which favor dissemination of conidia.

Apples leaves bearing abundantly sporulating scab lesions were placed in a glass tube through which a strong current of air was driven. A glass slide smeared with glycerine was held opposite the outlet of the tube in position to catch air-borne spores. Microscopic examinations following numerous repetitions of this test revealed only very small numbers of spores on the slides. Similar experiments with lesions on apple fruit gave like results. However, when these same lesions were treated with a fine mist of water applied by means of an atomizer, the droplets which accumulated contained conidia in great numbers. Similarly, when sections were cut from lesions with a dry razor and observed under the microscope, the addition of a droplet of water led to swelling of the conidiophores and immediate detachment of conidia. Furthermore, drippings collected during rains from scabby apple trees have shown an abundant content of conidia of *Venturia inaequalis*. These results, in conjunction with those from the air filtration experiments, indicate that no important dissemination of conidia is to be expected in the absence of water, though undoubtedly some spores are dislodged by wind-whipping of leaves, fruit, or branches, by contact with wind-blown particles, and in other minor ways. It appears, therefore, that the important agency for dissemination of these conidia is meteoric water moving under the influence of wind and gravitation.

GERMINATION TESTS

ASCOSPORES.—Germination tests with ascospores were made at frequent intervals throughout the period in which their natural discharge was observed in the field (May 18 to July 18). Fragments of leaves bearing perithecia were moistened and placed in such position that the spores would be discharged upon droplets of sterile distilled water on clean sterile glass slides in moist chambers or on plates of 2 per cent agar in water. The drops or plates were then placed in incubators at 18° to 25°C. In all cases naturally discharged ascospores showed a high percentage of vigorous germination, usually approximating 100 per cent. Germination tests of ascospores caught on the membranes gave like results. Some experiments conducted at constant temperatures ranging from 2° to 26° C. showed germination at all the temperatures tried, the optimum for germination and growth under these conditions being between 14° and 20° C.

CONIDIA.—Germination tests of conidia from fruit and leaf lesions were made at frequent intervals from their first appearance until late fall. The same media and incubators used for ascospore germination were employed. Abundant viable conidia could be found at any time during these experiments, but the percentage and vigor of germination varied greatly in individual tests. This is not surprising in view of the fact that mature conidia are much less protected than are ascospores and not so uniformly removed from the parent fungus when rain follows their maturity. Consequently, a sample of conidia, even when secured by touching a droplet of water borne in a wire loop to the sporulating surface of a scab lesion, is likely to be less uniform than a sample of naturally discharged ascospores.

SEASONAL DEVELOPMENT OF THE HOST PLANT

In relation to production and dissemination of spores and the seasonal development of the disease, the following brief notes on the seasonal development of the host plant are pertinent:

The unfolding of both vegetative and fruit buds was unusually late and slow in 1917. On May 7 the pink of the

petals of many fruit buds was evident, and by May 18 nearly all of the blossoms were open. On May 22 the largest leaves were about 2½ inches long, and petals were falling. The last leaves to develop appeared from about May 19 to 24, some variation occurring with varieties and individual trees. Most of the petals were off by May 25, and little leaf expansion occurred after June 2, when the first conidia of the season were observed. There was practically no leaf expansion after June 8 (except on "water sprouts").

SEASONAL DEVELOPMENT OF THE DISEASE

The seasonal development of the disease was studied by means of a series of bagging experiments, supplemented by daily observations in the orchard. No spray was used in this orchard. Each day from May 7 to June 19, six branches on at least two varieties were inclosed in large manila paper bags which were securely fastened in place with strong twine. In this way comparative records were kept of the development of the disease on branches which had been exposed to different infection periods. Many of the bags were broken [9] during storms, necessitating discarding a considerable number of branches from the experiment. Enough were left, however, to give some valuable data, a brief account of which follows.

None of the branches which were bagged before May 19 and on which the bags remained unbroken during the storms of May 30 and June 2 showed any evidence of infection throughout the period of observation, which ended on August 24. All branches bagged after May 22 developed infection, and with the exception of the last two leaves formed, which in some cases were put out after May 22, developed about as many leaf lesions as those which were continuously exposed to infection. Leaves of branches bagged after the rain on June 2 were infected in the same manner and to approximately the same extent as those of similar unbagged branches. This is of especial interest in view of the heavy ascospore discharge and favorable infection period of June 6–7.

The first leaf infection of the season was noted on June 2. This would allow a 14-day incubation period after the rain of May 19. This agrees with the results of field inoculation studies made in early spring in later years, in which the periods of incubation for leaf infection have commonly varied from 13 to 17 days. The records on ascospore dissemination and seasonal development of the disease are, therefore, in accord in placing the first ascospore discharge and infection of the season in the rainy period of May 19 to 22. The fact that no infection of significance occurred on leaves of branches which were bagged during the period May 19 to June 2 and subsequently exposed indicates that the leaves had become highly resistant to infection by the scab fungus by the latter date.

A striking suggestion of the variation in the susceptibility of leaves in relation to their stage of development was found in the number, distribution, and incubation periods of leaf lesions induced by natural infection. On June 2, when the first scab lesions of the season were observed, leaf expansion was almost finished. Lesions were clearly visible, in most cases, only on the upper surfaces of the apical leaves (for convenience called leaves No. 1) of shoots. Occasionally, early evidences of infection were apparent on the next leaf back, and within a day or two leaves No. 2 showed infection in about the same degree as No. 1. The development on leaves No. 3 and No. 4 was visible by June 8, while by June 12 to 15 small lesions were evident on many leaves No. 5 and No. 6 and rarely on No. 7. In late July and August the older leaves frequently showed traces of fungous growth on their lower surfaces but no definite lesions. The earlier infection thus appeared on the younger leaves and usually on the upper surfaces after relatively short incubation periods, while the latest infections to become evident appeared after prolonged incubation on the lower surfaces of the older leaves. Later field observations and field and greenhouse inoculation studies have confirmed the general outlines of these observations. They have shown, however, that there is a considerable range in the degree of these variations under different conditions. Under some conditions, for instance, only one or two leaves of a shoot will become infected from a given inoculation, while under others infection may develop on a half dozen or more.

The seasonal development of the disease on the fruit was much more difficult to follow in detail than on the leaves because of shedding induced by insect injury and other causes. The

[9] In later seasons the substitution of bags made of parchment paper obviated this difficulty.

first observation of fruit infection was made on June 8, when lesions were visible on the young fruits, calyx lobes, and pedicels. Daily field records showed that lesions continued to appear on the fruit throughout the period of observation, which ended on August 24. The results from the bagging work showed that the first fruit infection occurred during the rainy period of May 19 to 23. The most abundant infection and the largest lesions developed in the early part of the season. The fact that the later lesions are smaller suggests that the fruit, like the leaf, may develop resistance with age. Such resistance, however, appears to be developed in a much smaller degree, since fruit infection has been shown to appear late in the season or even in storage.

INOCULATION EXPERIMENTS

The life history studies were supplemented by some inoculation work in the orchard. Metal boxes, 28 by 12 by 10 inches, with tightly fitting, felt-lined covers, were constructed as infection chambers. A slit at one end of each box permitted a branch to enter and a tripod served as an adjustable support. The box was placed in position about the inoculated or control branch, the cover put in place, and the orifice through which the branch entered closed with plastic clay. A metal shelter about 4 inches above the box shaded it from the sun. When necessary the box was cooled by water.

Three types of shoots were selected for inoculation: (1) Those which had been bagged continuously since May 18 and were therefore free from infection, (2) those which had been exposed to infection throughout their development, and (3) those which developed during July ("water sprouts") and consequently bore young leaves. The inocula consisted of sterile-water suspensions of conidia from naturally infected leaves and from oatmeal-agar cultures. The experiments were perperformed in July and early August.

The chief results of these experiments may be summarized briefly. Thirty-six trials on disease-free shoots which had been bagged prior to May 18, and which bore no young leaves, gave no infection. In similar trials on shoots which had been exposed continuously to infection, there was no evidence that additional infection was induced. However, on branches which bore young leaves, abundant infection was induced on the young leaves provided the temperature was kept from running too high. While the exact maximum temperature for infection was not determined it appeared to be not much higher than 25° C. The maximum age of susceptible leaves is of course variable with conditions. In these tests it usually ranged between 12 and 15 days. These results are therefore in accord with those obtained in the life history and seasonal development studies.

SUMMARY

An effort was made to devise a satisfactory apparatus for determining the spore content of orchard air. Preliminary experiments were made with an electrical device. The results were promising, but the greater economy and simplicity of a mechanical filter led to its adoption for this work. It is believed, however, that the electrical method has potentialities of adaptation to problems of this type.

By wetting leaves freshly collected from the orchard it was shown that, in the vicinity of Madison, Wis., asci were capable of discharge under favorable conditions on May 7, 1917. Because of dry weather, however, the first natural discharge was delayed until May 19. Following this date ascospore discharges occurred during rains throughout the spring and early summer, the last recorded discharge occurring on July 18. The very heavy discharges, however, were limited to the period May 19 to June 7. After this time the discharges were relatively small.

The maximal concentration of ascospores of *Venturia inaequalis* in the orchard air was observed on May 21, when the average rate of catch for a period of 8½ hours was 1 ascospore to 0.4 liters of air. The wind velocity for this period varied from 14 to 23 miles an hour. The filter was 3 feet above the ground.

It was found that, when the asci were in condition to eject their spores, the presence of an adequate supply of water was the most important requisite for their discharge. In the experiments dew was not sufficient to induce discharges of consequence.

In cases where abundant asci were in condition to eject their spores in the presence of water, heavy discharge started soon after rain began and continued with continuous rain as long as the supply of ripe asci lasted. These periods of very heavy discharge lasted from 3 to 15 hours. Undoubtedly the duration of such periods varies widely with conditions.

Conidia of *Venturia inaequalis* were found in the air only during rain periods, and particularly when rain was accompanied by strong wind. Experiments showed that these conidia are very resistant to detachment from their conidiophores when dry, but quickly become detached in the presence of water. They are, therefore, disseminated chiefly in meteoric water acting under the influence of wind and gravitation.

Germination tests showed that practically all naturally discharged ascospores were vigorously viable. Conidia germinated with much less regularity. At no time after their appearance in the spring, however, was it difficult to find viable conidia in abundance.

The seasonal development of the host plant was unusually slow in the spring of 1917. In the Turville orchard most of the blossoms were open by May 18, and petal-fall was about complete on May 25. The last leaves to develop appeared from about May 19 to 24, and very little leaf expansion occurred after June 8.

Bagging experiments and orchard observations showed that most of the leaf infection of the season occurred during the period of May 19 to June 2, during which time all but one of the major ascospore discharges of the season occurred. The first observation of leaf lesions was made on June 2, 14 days after the first period of ascospore discharge. As the leaves approached maturity they became highly resistant to infection by the scab fungus.

Fruit infections were observed on June 8 and continued to appear throughout the period of observation, which ended on August 24. The records indicate that the first fruit infection occurred during the rainy period of May 19 to 23.

Successful leaf inoculation experiments were conducted in the orchard upon branches inclosed in specially constructed moist chambers. Infection from conidia applied in suspension in water was secured at will upon young leaves if fairly low temperatures (maximum at which infection occurred somewhat above 25°C.) were maintained. Old leaves were highly resistant to infection by *Venturia inaequalis*.

RELATION BETWEEN MORTALITY OF TREES ATTACKED BY THE SPRUCE BUDWORM (CACOECIA FUMIFERANA CLEM.) AND PREVIOUS GROWTH [1]

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INTRODUCTION

In a recent bulletin [3] reference is made to the local variation in mortality following spruce budworm defoliation. Several factors are considered as influencing the percentage of mortality, namely: Character of defoliation, available soil moisture, effects of severe winters, maturity, composition of the forest, and vigor of the trees.

But little data bearing directly on this last factor had been collected. In an earlier paper [4] the following quotation occurs: "The dying may be likened to a greatly accelerated natural thinning that takes place in a normal forest. It is suppressed or overmature trees that die first; younger stands suffer least."

In the first quoted bulletin [5] the writer makes the following statement: "The vigor of the stand at the time of the budworm attack largely determines the condition in which it will come through." The higher mortality in older forests as compared to younger forests is very striking, yet often spotted and by no means uniform; in second-growth stands, which suffer less, areas of high mortality are frequently found.

In comparing the rate of growth of trees dying on sample plots it was found that the slow-growing trees died first as well as those receiving heaviest defoliation, as illustrated by balsam in Table I.

A more detailed study of this feature was contemplated for the summer of 1922, but time did not permit its ful-

TABLE I.—*Radial increment in millimeters for 10-year period before budworm attack of trees dying at different periods*

[The first feeding occurred in 1918, the first mortality in 1920. Based on 2-acre plots containing over 350 trees, Lake Opasatika, Quebec]

	Summer 1920	Winter 1920-21	Summer 1921	Winter 1921-22	Summer 1922	Winter 1922-23
Increment in mm	8.2	10.5	10.9	11.7	14.0	15.4
Percentage of defoliation *	77	81	70	62	57	46
Average diameter, in inches	5.9	4.8	5.7	6.7	6.9	6.1

* Percentage of defoliation refers to old needles only at the end of the outbreak in 1921, or earlier in case of death. The new growth was completely destroyed each year from 1918 to 1921, inclusive.

[1] Received for publication April 22, 1924; issued June, 1925.

[2] This study was conducted by the writer as part of the 1923 program of work of the Division of Forest Insects, Dominion Entomological Branch, Ottawa, Canada.

The undertaking was greatly facilitated through the interested assistance of R. E. Balch and H. J. MacAloney, graduate students from Guelph College, Ontario, and the New York State College of Forestry, respectively. The collection of the data for Tables IV to IX and parts of Tables XII and XIII was made by the writer in person. On the remainder of the plots both the selection and tabulation were left entirely to the discretion of Balch and MacAloney. The compilation of the data was made by all three members of the crew. J. D. Tothill also assisted in taking some plots.

W. G. Wright, in charge of research work for the Dominion forestry branch, Ottawa, kindly assisted with suggestions concerning the tabulation of the data. E. N. Munns, Raphael Zon, and S. T. Dana offered valuable assistance through constructive suggestions on the preliminary manuscript.

This presentation represents a considerable condensation of the original manuscript, copies of which, with complete tables and data, are filed at the Bureau of Entomology and Forest Service, Washington, D. C., the Entomological Branch, Ottawa, and the New England Forest Service Experiment Station, Amherst, Mass.

[3] SWAINE, J. M., and CRAIGHEAD, F. C. STUDIES ON THE SPRUCE BUDWORM. Canada Dept. Agr. Bul. (n. s.) 37, 92 p., illus. 1924.

[4] CRAIGHEAD, F. C. BUDWORM INFESTATION VS. PULPWOOD PRODUCTION. 2. Amer. Paper and Pulp Assoc., Woodlands Sect. Ser. Proc., Ann. Meeting 2: 8-10, 1922.

[5] Footnote 3, Part II of this bulletin.

fillment. During the summer of 1923 data on a series of plots in various forest types were taken near Bathurst, New Brunswick, and Metis Lake, Quebec.

The studies were concentrated on second-growth forests. Mature forests, where the mortality was much higher, were avoided, owing to the complication of other factors, such as periodic cuttings. It was also deemed advisable to put more time on the second-growth stands, since such results would be more directly applicable to forest conditions of the future.

EXPLANATION OF DATA

In the softwood forests one-tenth acre plots were used. It was found that this size suited the purpose better, since the variation in injury was quite local and spotted. The percentages of balsam, red spruce, and white spruce, and the basal area and average diameter are based on the total of softwoods only; the percentage of hardwoods is that of the total number of trees on the plot. The average diameter was computed from the basal area. All trees from 3-inch diameter breasthigh and up were tallied and all data computed on this basis. This naturally gives a low expression for average diameter.

The percentage of mortality was computed on the basis of number of trees rather than on volume or basal area. No volume tables were available for these small diameters, and it was thought that percentages by trees would give a better expression of the effects of defoliation on the plots, especially the suppressed ones. In cases where the injury is low, it would often be quite negligible if computed in volume.

All increments are expressed in millimeters, showing the radius for a 10-year period unless otherwise stated. In each plot a varying number of cores were taken at breastheight (by means of an accretion borer), depending on the percentage of the species and number of each dead. At least 10 cores from dead and living trees of each species were secured, or, if the number of trees in such classes was small, at least half were taken. No definite method of selecting trees was used; the plot was gone through and all trees taken as encountered selected from all diameter classes. In computing the average rate of growth for each species on the plot, averages were first obtained from the selected cores for both dead and living trees. These averages were multiplied by the total number of dead and living trees, respectively, added and divided by the total number of that species on the plot. On the first few plots the average rate of growth for each species was determined from dominant trees only, average-diameter trees, all trees or the method described. The variation between different plots as expressed by dominant trees and average-diameter trees was considerable, but that between the last two methods was so slight that the one involving less time was adopted.

The 10-year period previous to budworm attack was counted back from the enlarged ring produced by the defoliation. This is very marked on balsam. In spruce, where the enlargement is less pronounced, the year of budworm attack (previously determined for the region) can be counted back from the last ring formed, but this is not reliable for balsam, owing to the fact that two or three rings may fail to form on parts of the circumference and not show on the increment core. On dead or dying trees, both spruce and balsam, the enlarged ring must be utilized for orientation, since from two to four rings may fail to form previous to death.

CHARACTER OF BUDWORM DEFOLIATION [6]

A brief description of the character of the budworm feeding will help to explain certain results of the tabulated data. Previous investigations have shown that the severity of defoliation is the primary cause of death of the trees through inhibiting normal physiological functions.

The degree of defoliation of the spruce and balsam is chiefly due to the variation in development of the new growth, to the migratory habits of the larvae, and to the fact that the old foliage of spruce is not consumed by the larvae. For normal development, the young caterpillars require new foliage as food. They begin feeding at the time the balsam and white-spruce buds open. During epidemics their abundance is such that the new growth of balsam is consumed by the time the caterpillars are half grown; in the later instars they are able to subsist on the old needles and consume up to 100 per cent of these. White spruce furnishes a greater abundance of new growth, so that rarely do the larvae consume all of it before the needles harden. This hardening of the needles takes place about the time the caterpillars are half grown, causing

[6] For more detail see reference, footnote 3.

them to migrate in search of more succulent food. It is possible that slower-growing white-spruce trees putting out a less quantity of new growth are entirely defoliated of the current year's needles before they harden. Red spruce, the buds of which open 10 to 12 days later than balsam and white spruce (shortly before the larvae begin migrating), furnishes a second supply of succulent needles at a time when the larvae are most voracious; consequently much of it is consumed.

The density of the stand affects the degree of defoliation in that migrating larvae have a better chance of falling on other food before striking the ground, from which they can not regain the trees. As a result of this migrating habit of the larvae no trees, even balsam, standing in the open are defoliated of more than their new growth.

Higher percentages of balsam and white spruce encourage to a certain extent heavier defoliation on account of the greater supply of desirable food (early new growth), which enables the larvae to develop rapidly in their early stages.

Balsam is always more severely defoliated than white or red spruce, since the old needles are also consumed; consequently, the feeding is less uniform and offers an explanation for lack of correlation between mortality and rate of growth under conditions of severe feeding. White and red spruce are defoliated only of the new growth, white usually to a lesser degree than red, and both more uniformly than balsam. The amount of defoliation on the balsam sample plots at Lake Opasatika, Quebec, from 1918 to 1921 is shown in Table II.

Dominant trees, especially those highest in the stand, are less severely defoliated than those beneath, owing to the migrating habits of the larvae. Table III illustrates this feature on the balsam sample plots at Lake Opasatika. The figures apply only to old foliage, since all the new growth was consumed each year.

Thus the understory, which at the same time is composed of slower-growing trees, receives more defoliation. This does not apply to light outbreaks when, with abundance of food, the larvae do not migrate.

SECOND-GROWTH SOFTWOOD AND BUDWORM MORTALITY

Two widely separated areas were considered in these studies, centering about Bathurst, New Brunswick, and Metis Lake, Quebec.

The Bathurst plots were located about 15 to 20 miles south of Bathurst, on the Tabusintac drainage. The soil is of a light sandy character, formed from the millstone grit of the middle Carboniferous. It is fairly thin and subject to excessive drying out, except on the hardwood ridges, where it is much deeper and of a loamy character. It fairly well characterizes the Miramichi watershed, noted for its spruce forests.

The other series of plots were taken on the south shore of the St. Lawrence River near the height of land between the Metis and Patapedia Rivers, on a seigniory of Price Brothers at Metis Lake, Quebec. It is essentially a softwood region, only scattered yellow and white birch occurring, except on the tops of the higher hills. Some 40 years ago the area was very heavily cut over, so that the present stand averages about 75 years of age. A few older trees occurred on many plots. An attempt was made to secure the plots in more uniform younger growth and at the same time to select plots which showed higher mortality. Consequently, these figures are not quite typical of the area.

In the vicinity of Bathurst 40 plots were tallied in practically pure softwood stands. An attempt was made to group these by series conforming to site qualities, types, and age classes. Tabulation of 24 plots is given occurring on a 65-year burn representing three sites of the spruce flat type. The remaining plots were more scattered in

TABLE II.—*Percentage of trees in arbitrary defoliation classes*

Degree of defoliation	100	90	75	50	25	10
Percentage of trees in class	5.6	10.6	16.9	33.2	23.8	9.9

TABLE III.—*Percentage of defoliation by diameter classes*

Diameter breasthigh, inches	2	3	4	5	6	7	8	9	10	11	12
Number of trees	4	47	65	31	57	54	44	30	19	11	11
Average defoliation, per cent	54	77	66	64	51	46	42	29	28	28	14

various age classes up to 100 years and in jack pine and spruce swamp types. In general they conformed to the results here shown, but the number of plots on each condition was not sufficient to be conclusive, and consequently they are not included. Altogether, measurements were recorded from over 3,000 increment cores.

The defoliation on these plots was very severe, and all evidence indicates that it was quite uniform for the entire area.

The Metis plots, Tables X and XI, were chosen because of light budworm feeding for comparison with the heavy feeding in the Tabusintac area. The attack began in 1913. Although the percentage of balsam was high, the feeding apparently only lasted, with any degree of severity, for one year. This was reported from observations of the company officers who frequently visit the place during the summer. It is also further substantiated by the more rapid recovery, or greater increment for the 10-year period following defoliation, as compared to any other regions of Quebec and New Brunswick which have been studied. (See Table XVIII.)

The reason [7] for the light feeding, which is certainly due to the early dying out of the infestation, has been unexplained. It may have resulted from weather conditions. A similar state prevails eastward throughout the Gaspe Peninsula.

This series of plots was selected and tallied by Balch and MacAloney. The writer had previously visited the region on two occasions, but did not go over the present work. No attempt was made to segregate these plots by types or quality sites.

This region as a whole is characterized by preponderance of white spruce over red spruce.

TABLE IV.—*Composition of plots, Bathurst, New Brunswick*

No.	Balsam	Red spruce	White spruce	Hardwoods	Number of trees	Average diameter [a]	Basal area	Height	Largest trees [b]	
									Number	D. b. h.
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*		*Inches*
7a	7.1	92.9	0	0	70	5.3	10.68	45	6	8
7	0.0	100.0	0	0	108	5.7	19.02	55	3	9
8	3.8	96.2	0	0	105	5.6	18.40	50	2	10
9	2.1	97.9	0	0	96	5.7	17.21	55	3	9
7c	13.2	86.8	0	0	106	5.9	20.31	60	2	10

[a] Includes trees 3 inches d. b. h. and over.
[b] The expression "Largest trees, d. b. h.," indicates the diameter class of the largest trees, and "Number," the number of trees in that class.

TABLE V.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality, of trees in plots of Table IV* [a]

No.	Dead spruce	Radial increment of spruce		
		Living	Dead	All
	Per cent			
7a	13.8	8.6	5.8	8.2
7	14.7	8.4	7.3	8.2
8	21.7	7.3	5.8	7.0
9	50.0	6.7	6.2	6.4
7c	49.0	5.5	4.4	5.0
Average		7.6 (94)	5.8 (54)	6.9

[a] Figures in parentheses refer to number of measurements.

[7] Heavy rainstorms at the time of opening of the buds and severe frosts, killing the new growth, have both been reported by Tothill and Craighead as causing high mortality in the budworm larvae.

TABLE VI.—*Composition of plots, Bathurst, New Brunswick*

No.	Balsam	Red spruce	White spruce	Hardwoods	Number of trees	Average diameter	Basal area	Height	Largest trees	
									Number	D. b. h.
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*		*Inches*
3d	12.6	67.2	21.2	10.7	67	6.8	16.75	60	5	11
3c	4.8	75.0	20.2	1.2	84	6.8	20.49	60	2	11
3b	7.7	62.6	29.7	1.0	91	7.1	24.87	60	2	14
3a	30.2	52.4	17.4	1.5	63	8.0	24.17	70	1	15
9b	8.2	91.8	0	15.5	98	6.0	19.49	57	3	10
9a	5.8	94.2	0	8.4	87	6.1	17.77	55	2	10
6a	5.5	67.5	27.0	0	111	6.3	24.35	56	2	11
6b	1.2	67.4	31.4	11.9	89	6.1	18.20	56	3	10

TABLE VII.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality of trees in plots of Table VI* [a]

No.	Balsam, increment				Red spruce, increment				White spruce, increment				All spruce	
	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Increment
	P. ct.	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*
3d	14.3	9.7	------	9.7	2.2	10.6	------	10.6	7.1	13.3	------	13.3	3.4	11.2
3c	50.0	7.0	------	7.0	3.2	10.6	6.0	10.4	35.3	11.4	4.6	9.0	10.0	10.3
3b	28.5	12.0	3.0	9.4	10.8	9.9	------	9.9	44.4	10.0	5.9	8.1	15.4	9.3
3a	100.0	------	9.2	9.2	15.1	9.4	7.2	9.1	36.4	8.9	6.0	7.8	20.5	8.8
9b	50.0	16.0	17.0	16.5	23.3	9.0	7.2	8.6	------	------	------	------	23.3	8.6
9a	100.0	------	7.7	7.7	40.3	9.0	6.2	7.8	------	------	------	------	40.3	7.8
6a	33.3	------	------	------	40.0	8.9	7.0	8.1	66.6	8.6	4.9	6.1	47.6	7.6
6b	100.0	------	------	------	41.7	8.8	5.5	7.4	53.7	10.0	2.7	6.1	45.4	7.0
Average	------	11.6 (12)	9.4 (16)	10.4	------	9.5 (118)	6.5 (54)	8.5	------	10.5 (40)	4.8 (29)	8.1	------	------

[a] Figures in parentheses refer to number of measurements.

TABLE VIII.—*Composition of plots, Bathurst, New Brunswick*

Plot No.	Balsam	Red spruce	White spruce	Hardwood	Number of trees	Average diameter	Basal area	Height	Largest trees (65 years)	
									Number	D. b. h.
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*		*Inches*
18	21.4	28.6	50.0	0	57	6.9	15.42	70	2	15
7b	53.2	42.0	4.8	26.2	62	6.0	12.78	65	1	12
17a	1.2	73.2	25.6	0	82	7.0	22.11	65	2	12
16	11.6	26.8	61.6	0	112	6.5	25.61	65	3	11
17	22.2	29.6	48.2	0	54	8.4	20.75	68	3	12
15	15.6	31.1	53.3	2	90	7.9	26.32	65	3	10
7	30.3	63.2	6.5	10.6	76	6.2	15.96	60	2	10
7a	39.5	51.1	9.4	2.3	86	6.5	19.97	61	2	11
14a	26.4	46.1	26.4	4.7	121	5.8	22.37	60	6	9
13	15.6	6.9	77.4	0	116	7.0	31.08	60	1	13
14	57.6	5.6	36.8	0	126	5.8	23.46	60	1	10

TABLE IX.—*Radial increment in millimeters in d. b. h. from 1903 to 1912, inclusive, and mortality in trees of plots of Table VIII* [a]

Plot No.	Balsam, increment				Red spruce, increment				White spruce, increment				All spruce	
	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Increment, all
	P. ct.	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*
18	0	15.1	.....	15.1	0	9.4	.....	9.4	0	9.7	.....	9.7	0	9.6
7b	64.2	11.7	7.8	8.9	3.8	9.1	13.0	9.3	33.3	12.7	2.0	9.8	4.8	9.2
17a	0	.....	.....	.....	5.0	11.9	13.3	11.9	14.3	6.2	4.5	6.0	7.4	8.0
16	77.0	.....	10.6	10.6	16.6	10.6	7.8	10.1	29.0	7.1	3.1	5.9	25.2	7.2
17	91.7	.....	9.1	9.1	43.7	6.3	7.1	6.0	30.8	8.1	4.6	7.0	35.7	6.9
15	100.0	.....	6.1	6.1	28.6	7.0	7.2	7.1	45.8	9.2	4.8	7.2	39.5	7.1
7	91.4	.....	7.0	7.0	40.0	7.2	7.6	7.3	60.0	5.0	2.0	3.3	41.5	7.0
7a	97.1	.....	9.7	9.7	52.3	6.9	6.6	6.7	50.0	5.0	2.5	3.7	52.0	6.3
14a	100.0	.....	6.2	6.2	50.9	7.6	6.3	6.9	65.6	5.0	3.4	4.0	56.2	6.0
13	61.1	.....	7.7	7.7	50.0	10.7	8.5	9.6	75.3	6.8	4.3	4.9	73.2	5.3
14	100.0	.....	6.2	6.2	71.4	.....	.....	.....	80.4	6.6	5.3	5.4	79.2	5.4
Average	.....	13.5 (11)	7.4 (68)	8.3	.....	8.9 (91)	7.5 (55)	8.3	.....	7.5 (68)	4.2 (61)	5.9	.....	.....

[a] Figures in parentheses refer to number of measurements.

TABLE X.—*Composition of plots, Metis Lake, Quebec* [a]

Balsam	Dead balsam	Red spruce	Dead red spruce	White spruce	Dead white spruce	Hardwoods	Average number trees per plot	Average diameter	Average basal area	Average height	Average age, in years
Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*	
79.1	39.1	5.3	0	15.6	11.6	5.7	88.8	6.7	21.14	60	70

[a] Based on 33 plots.

TABLE XI.—*Radial increment in millimeters from 1903 to 1912, inclusive, and mortality of trees in plots of Table X. Plots averaged by groups in percentage classes* [a]

Dead	Balsam, increment			White spruce, increment			Red spruce, [b] increment
	Living	Dead	All	Living	Dead	All	Living
Per cent	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
0 to 5	12.4	2.0	12.3	11.8	3.2	11.1	.....
5 to 15	10.6	5.0	10.4	10.6	5.3	10.0	.....
15 to 25	10.2	5.0	9.2	11.2	5.8	10.0	.....
25 to 35	9.5	5.3	8.0	9.3	3.5	7.6	.....
35 to 45	8.3	5.0	6.9	.....	.....	.....	.....
45 to 55	8.8	4.4	6.7	7.5	2.5	5.0	.....
55 to 65	8.1	5.2	6.3	7.5	3.5	5.1	.....
65 to 75	7.8	4.4	5.2	.....	.....	.....	.....
Average	9.0 (507)	5.0 (335)	7.4	9.6 (220)	4.3 (43)	8.8	8.4 (77)

[a] Figures in parentheses refer to number of measurements.
[b] Too few trees per plot to be considered.

From the data collected and from general observations made, certain conclusions are drawn concerning budworm mortality and silvicultural characteristics of balsam fir, white spruce, and red spruce. These, since they are based on limited areas, are presented as tentative and with the object of inviting criticism and further investigations from the foresters and entomologists familiar with the budworm infested regions. It is fully realized that with so many factors to consider it is practically impossible to make comparisons of conditions alike except for one factor and that the results brought out in this paper are largely suggestive rather than conclusive.

There is a certain correlation between vigor of the stands (as expressed by increment at breastheight) at the time of defoliation and budworm mortality such that it can be said the more rapid the rate of growth the lower is the mortality resulting from defoliation. This expression of the probable effects of budworm feeding is a more tangible quantity than any of the other factors heretofore considered, and should serve as a practical basis for preventive measures through proper silvicultural practices which maintain rapid growth.

There may be different minimum rates of growth, entailing immunity for different sites, types, and age classes, indicating the necessity of further investigation in conditions other than those discussed here.

All the data bearing on density and basal area were plotted with the assistance of E. N. Munns, but no correlation was found. Percentage of balsam in the mixture was likewise considered, but this bore no relation to the mortality of the spruce.

The correlation between rate of growth and mortality for balsam is not at all regular for the Bathurst series (figs. 1 and 2, Tables IV to IX), owing to the severe infestation in that region, resulting in complete defoliation of many trees. From 75 to 100 per cent defoliation will kill even very vigorous trees. The correlation for the white and red spruces is much more regular. These trees are more uniformly defoliated, only the new growth being eaten for three to four years; that for combined red and white spruce is still more regular, the explanation being doubtful unless it is because of the greater number of trees averaged. On the Metis area (Tables X and XI, fig. 2) the correlation between rate of growth and mortality for balsam is quite regular; that for white spruce shows very little correlation. Here the dead spruce trees are all small suppressed trees of the understory (see Table XVII, mortality by diameter classes) as compared with the Bathurst spruce and Metis balsam. In fact, so few spruces are dead that the entire mortality in these trees is considered to be that of a normal forest and gives a series of plots for contrast to conditions at Bathurst.

The diameter class tallies by species as well as by observation on the stumps of many felled trees indicate that

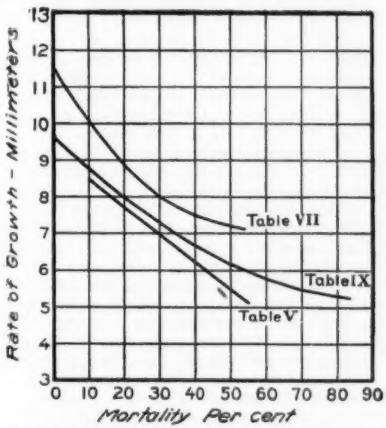

FIG. 1.—Diagram illustrating rate of growth of spruce and mortality resulting from spruce budworm defoliation as recorded in Tables V, VII, and IX

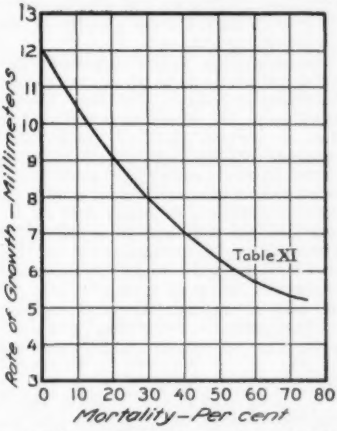

FIG. 2.—Diagram illustrating rate of growth of fir and mortality resulting from spruce budworm defoliation as recorded in Table XI

second-growth white spruce grows faster than either red spruce or balsam during the first 40 to 50 years, but after certain densities are reached its growth is retarded and it can not compete as well as red spruce. Considering the higher mortality of white spruce compared to that of red spruce in 60-year stands, the relatively lighter feeding (excepting the possibility that slow-growing trees may be subject to heavier defoliation) and the average slower rate of growth for dying trees, it may be considered that white spruce is more susceptible to budworm attacks than red spruce. This suggests that it should be encouraged only when good vigor can be maintained. Also a higher percentage of white spruce, like balsam, enhances heavier average defoliation of adjacent red spruce owing to the abundant food supply in the early feeding stages.

Balsam is the most susceptible under all conditions, since it is completely defoliated in heavy outbreaks. On the other hand, the Metis plots indicate, as well as certain Bathurst plots, that vigorous stands are more immune. Since balsam is a very fast grower, and reproduces abundantly and under adverse conditions, it might well be encouraged for the first 30 to 40 years. The only argument against it is that over wide areas it is considered the most important factor in giving impetus to an outbreak, though balsam is probably no more effective in this respect than is a mixture of white spruce and red spruce.

HARDWOOD MIXTURES AND BUDWORM MORTALITY

Two series of plots (Tables XII, XIII, XIV, and XV) were taken in hardwood mixtures. The object was to obtain some idea of the effect of budworm feeding on the free and overtopped softwoods in such stands. It has been held by several investigators that the mortality in hardwood mixtures is always considerably lower than in pure softwoods.

Two types were considered. Tables XII and XIII summarize 19 plots, totaling 3 acres, in a 60 to 65 year birch-poplar type on the same area as the softwood plots of Tables IV, VI, and VIII. Tables XIV and XV summarize 11 plots, totaling 3½ acres, in a northern hardwood type on the Bathurst area. This area has been subjected to periodic cuttings in the past. No white spruce occurred. The largest diameter for softwoods was about 20 inches. These plots were located on the top of a low ridge on the best growing site in the region.

Percentage expressions are the same as for previous plots except that total percentage of hardwoods is calculated on the basis of all trees on the plots. This expression was not considered a fair indication of the hardwood canopy, so the percentage of overtopped softwoods is used as indicating the amount of hardwood canopy. Two classes of softwoods were considered—overtopped, those whose terminals were under the softwood canopy, and free, those

TABLE XII.—*Birch and poplar type, composition of plots*

Balsam	Balsam, overtopped	Red spruce	Red spruce, overtopped	White spruce	White spruce, overtopped	Softwoods, overtopped	White birch	Poplar	Total hardwoods	Average number trees per ⅓ acre	Softwood, average diameter	Basal area, ⅓ acre
P. ct. 34.2	*P. ct.* 39.2	*P. ct.* 50.3	*P. ct.* 38.6	*P. ct.* 15.4	*P. ct.* 32.0	*P. ct.* 60.7	*P. ct.* 70.7	*P. ct.* 29.3	*P. ct.* 24.2	70	*In.* 6.3	*Sq. ft.* 15.34

TABLE XIII.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality of trees in plots of Table XII*

	Balsam, increment				Red spruce, increment				White spruce, increment			
	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Living	Dead	All
	P. ct.	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*
Free	78.5	17.1	10.9	11.9	23.6	10.8	10.7	10 8	18.7	11.9	6.4	10.9
Overtopped	38.8	6.8	6.6	6.7	7.6	8.7	7.4	8.6	21.8	8.9	6.4	8.4
Average	228 trees			8.2	293 trees			9.5	117 trees			9.7

TABLE XIV.—*Northern hardwood type, composition of plots*

Balsam	Balsam, overtopped	Red spruce	Red spruce, overtopped	White spruce	White spruce, overtopped	Softwoods, overtopped	Yellow birch	Maple	Beech	Hemlock	Total hardwoods	Number trees, ⅓ acre	Softwood, average diameter	Basal area, ⅓ acre
P. ct. 46.7	*P. ct.* 38.8	*P. ct.* 53.3	*P. ct.* 35.7	*P. ct.* 0	*P. ct.* -------	*P. ct.* 59.7	*P. ct.* 20	*P. ct.* 15.3	*P. ct.* 64.7	*P. ct.* 0.4	*P. ct.* 27.4	32.2	*In.* 6.8	*Sq. ft.* 7.20

TABLE XV.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality of trees in plots of Table XIV*

	Balsam, increment				Red spruce, increment			
	Dead	Living	Dead	All	Dead	Living	Dead	All
	Per cent	*Mm.*	*Mm.*	*Mm.*	*Per cent*	*Mm.*	*Mm.*	*Mm.*
Free	58.8	17.7	14.9	16.3	39.6	18.4	22.1	19.8
Overtopped	9.9	10.7	7.5	10.1	5.4	10.0	5.5	8.2
Average	206 trees			13.2	234 trees			14.6

whose terminals were above the canopy or standing in an opening in the hardwood foliage. Since no comparison of individual plots was contemplated, the tabulated data are a summary for all plots.

The mortality of four softwood series at Bathurst and Metis (Tables IV to XI) and two hardwood series (Tables XII to XV) is compared in Table XVI. The hardwood mixtures show considerably less mortality for white spruce and red spruce, though this is not so marked for balsam. If the percentages of mortality among the free and overtopped trees are compared, it is seen that the reduction in mortality is chiefly due to the overtopped trees.

In these series of plots the rate of growth for the overtopped trees is considerably lower than for free trees, so this factor would tend to increase mortality if it were not overbalanced by the protection offered by the hardwood canopy. The average rate of growth for the species of softwood trees in the hardwood series (Tables XII, XIII, XIV, and XV) is lower for balsam and higher for both red and white spruce than in the preceding softwood series.

In the northern hardwood type (Tables XIV and XV) the average rate of growth for all balsam (13.2) and red spruce (14.6) is considerably higher than for any other series of plots. Likewise the mortality of the overtopped softwoods is lower and shows a greater contrast to the free dead softwoods. The total mortality for balsam is lower than in the birch-poplar type (Tables XII and XIII), while that for red spruce is higher. It so happens that in these plots the rate of growth for the dead red spruce is higher than for the living. This discrepancy may be due to the method of tabulating free and overtopped trees, which does not properly group the trees according to the relative amount of defoliation. Balch recognized this, adding the following note on one plot:

> The free living red spruce are smaller than the free dead red spruce. The free living are in many cases partly overtopped, the free dead generally almost entirely free. Thus the percentage of canopy seems a greater factor than rate of growth.

These free living dead trees are thus subjected to a greater foliage exposure, and consequently to heavier defoliation, than those which survived and are partly overtopped, though classed as free because the terminal was free.

The lower percentage of mortality of the softwoods in hardwood mixtures is entirely a matter of protection by the hardwood foliage. This protective effect is due to concealment of overtopped softwoods from the ovipositing moths and the lessened chance of migrating larvae falling on the softwoods.

The mortality among the free softwoods of hardwood mixtures is about the same or slightly higher for balsam than for the pure softwood. This substantiated many observations in the field where high mortality was observed, especially in older hardwood mixtures when the hardwoods were

becoming decadent, and suggests that the conifers, after years of competition in hardwood mixtures, are even less resistant than those in softwood stands.

Some indication of the relative ability of these trees to withstand severe competition is obtained from the tables summarizing the hardwood plots (Tables XII, XIII, XIV, and XV) and that of the mortality by diameter classes (Table XVII), suggesting that white spruce is least resistant as indicated by the higher mortality of the overtopped trees of the birch-poplar type (Tables XII and XIII), and the higher percentage of mortality in the 3, 4, and 5 inch diameter classes of softwood types. The same tables indicate that balsam can withstand more competition than white spruce and that red spruce is the most resistant species.

MORTALITY BY DIAMETER CLASSES WITH SEVERE AND LIGHT DEFOLIATION

The following table of the tree mortality (Table XVII) of three series of plots tabulated by diameter classes is given to show the relative effects of different degrees of caterpillar feeding on the various softwoods concerned and how such feeding affects mortality in the larger and smaller tree classes.

decreasing in the smaller and larger trees; in white spruce highest mortality occurs in the smaller-diameter classes, decreasing in the higher diameters.

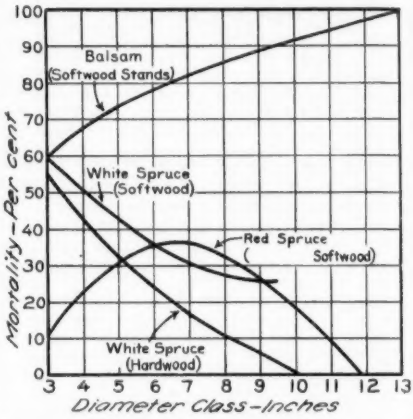

FIG. 3.—Mortality of spruce and fir in various forest types resulting from severe defoliation by the spruce budworm, shown by diameter classes. The white spruce (hardwood) are free

Considering the same combination but with lighter feeding (fig. 4) as occurred at Metis, where the red and white spruce are very little affected (in fact, it might be questioned if

TABLE XVI.—*Percentages showing softwood composition before budworm attack and total mortality for each series of plots*

	Balsam		Red spruce		White spruce	
	Mortality	Composition	Mortality	Composition	Mortality	Composition
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Softwoods from Tables IV and V	5.2	66.2	94.8	29.8		
Softwoods from Tables VI and VII	9.5	59.5	72.1	22.1	18.4	40.6
Softwoods from Tables VIII and IX	27.4	60.2	33.3	27.9	39.2	37.3
Softwoods from Tables X and XI	79.1	39.1	5.3	5.5	15.6	11.6
Hardwoods from Tables XII and XIII	34.2	53.0	50.3	13.4	15.4	20.2
Free trees (alone)	60.8	78.5	61.3	23.6	68.0	18.7
Overtopped trees (alone)	39.2	38.8	38.6	7.6	32.0	21.8
Hardwoods from Tables XIV and XV	46.7	27.2	53.3	20.5		
Free trees (alone)	61.2	58.8	64.3	39.6		
Overtopped trees (alone)	38.8	9.9	35.7	5.4		

Considering the mortality of the three species of trees concerned by diameter classes and under conditions of heavy defoliation (fig. 3) (such as is represented by softwood stands of Tables VIII and IX) it is shown that in balsam mortality is relatively high in all classes, but increases with diameter; in red spruce highest mortality occurs in the middle-diameter classes, mortality in these two species can be attributed to other than natural thinning), we find that in balsam greatest mortality occurs in smaller-diameter classes, decreasing with larger diameters; in red spruce all mortality occurs in low-diameter classes below 6 inches; in white spruce all mortality occurs in low-diameter classes below 6 inches.

Considering mortality in the hardwood plots, Tables XII and XIII, where defoliation was severe on the free trees and relatively lighter in the overtopped trees, it is shown that balsam mortality of free trees is similar to that in softwoods (as illustrated in fig. 3); among the overtopped trees it falls quite regularly from the smaller diameters to the higher. Red spruce mortality among the free trees is similar to that of softwood stands (as illustrated in fig. 3); among the overtopped trees it falls quite regularly from the smaller diameters to the higher. White spruce mortality among the free trees falls rapidly as diameter increases and is lower than white spruce in the softwoods; among the overtopped trees the mortality falls similarly to that of red spruce, although it is higher in all diameters.

It might be assumed that the correlation drawn between rate of growth and mortality is not conclusive from the fact that the position of the tree in relation to the crown influences the amount of defoliation (intermediate and suppressed trees receive relatively more defoliation in severe epidemics), and degree of defoliation is no doubt the most important factor causing death. In other words, may not the higher mortality of slower-growing trees be a result of their position?

This is clearly not the case with balsam, which is subject to heavy defoliation, since greater mortality occurs as diameter increases, and there was found to be no correlation between rate of growth and mortality for such heavily defoliated balsam.

With red spruce receiving severe defoliation both in pure softwoods and free trees in hardwood stands the greatest mortality occurs between the diameter classes 6 and 8 inches, which trees in these 60-year stands would be largely either dominants or codominants.

In the case of white spruce receiving heavy defoliation, greatest mortality does occur in the lower-diameter classes 3, 4, and 5 inches, and here it is impossible to decide how much of the mortality is due to position, suppression, or budworm defoliation. In hardwood stands (Table XVII) greater total mortality occurs in the overtopped trees, which receive less defoliation, than in the free trees. On the other hand, with lighter feeding, mortality in all species is greater in the lower-diameter classes, which, of course, on the whole are slower-growing trees, but here again in balsam, which receives more defoliation, the mortality persists well up into high-diameter classes (both in softwood stands and overtopped trees in mixed hardwood stands) where many of the trees in softwood stands would be dominants and codominants.

It is not intended to argue that position does not have a certain effect on mortality, yet it seems impossible this long after the budworm feeding to determine just what weight can be attributed to it.[3] Referring to Table I, it will be seen that there is little difference in the average diameter of trees dying over a three-year period, while rate of growth and percentage of defoliation show regularly increasing or decreasing values in respect to time of death.

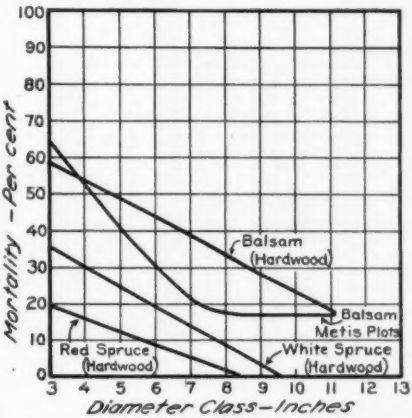

FIG. 4.—Mortality of spruce and fir in various forest types resulting from light defoliation in hardwood mixtures by the spruce budworm, shown by diameter classes. The balsam (hard wood), red spruce, and white spruce are overtopped

COMPARISON OF RADIAL INCREMENT OF SOFTWOODS BEFORE AND AFTER BUDWORM ATTACK

These series of measurements (Table XVIII and fig. 5) were taken to compare the rate of growth of partially defoliated trees before and after the budworm attack. The trees were selected promiscuously over the areas on which the foregoing plots were taken. Only dominant or codominant trees

[3] The degree of feeding on individual trees could not be determined at the time of this study.

TABLE XVII.—*Mortality by diameter classes (in percentages)*

SEVERE FEEDING

Tree species and association	3	4	5	6	7	8	9	10	11	12	13
Balsam:											
Softwoods of Tables VIII and IX	40	71	75	83	79	96	83	67	0	100	0
Free trees in hardswoods, Tables XII and XIII	50	62	82	79	70	93	79	92	90	83	100
Red spruce:											
Softwoods of Tables VIII and IX	19	9	36	39	33	32	29	7	11	0	0
Free trees in hardwoods, Tables XII and XIII	20	31	23	22	36	26	20	14	0	0	0
White spruce:											
Softwoods of Tables VIII and IX	48	60	56	12	32	32	21	32	21	0	0
Free trees in hardwoods, Tables XII and XIII	------	54	24	30	17	13	0	0	0	0	0

LIGHTER DEGREES OF FEEDING

Balsam:											
Softwoods of Tables X and XI	55	71	48	33	19	21	16	21	18	0	0
Overtopped trees in hardwoods, Tables XII and XIII	30	37	49	44	42	30	18	29	------	------	------
Red Spruce:											
Softwoods of Tables X and XI	0	25	3	0	0	0	0	0	0	0	0
Overtopped trees in hardwoods, Tables XII and XIII	57	11	8	9	12	8	0	0	------	------	------
White spruce:											
Softwoods of Tables X and XI	39	35	24	3	0	0	0	0	0	0	0
Overtopped trees in hardwoods of Tables XII and XIII	33	29	33	17	18	8	0	0	0	------	------

TABLE XVIII.—*Rate of growth before and after budworm attack*

FROM SPRUCE FLAT TYPE, BATHURST, NEW BRUNSWICK

	Balsam				Red spruce				White spruce			
	10 years before	5 years before	10 years after	Number of trees	10 years before	5 years before	10 years after	Number of trees	10 years before	5 years before	10 years after	Number of trees
	Mm.	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*	
Living [a]	19.9	9.2	11.0	85	12.2	5.7	7.2	88	12.3	5.6	6.5	89
Dead [b]	12.2	5.4	------	199	9.3	4.4	------	192	5.5	2.2	------	133

FROM NORTHERN HARDWOOD TYPE, BATHURST, NEW BRUNSWICK

Living	18.9	10.4	12.5	90	19.8	10.0	13.0	116	------	------	------	------
Dead	18.7	9.9	------	63	16.6	9.6	------	76	------	------	------	------

FROM METIS LAKE, QUEBEC

Living	11.5	5.3	7.8	16	------	------	------	------	------	------	------	------

[a] From trees which recovered.
[b] From trees which died in 1922 from effects of defoliation

were bored. For this reason they can hardly be used to express the rate of growth of the regions as a whole so well as can the plot increments.

On the softwood plots the rate of growth for the 10-year period following first feeding (i. e., including 4-year feeding period and 6 years' recovery) was only about one-half that of the previous 10 years, while in the northern hardwood type this increment was about two-thirds the previous 10-year period. This may be due to greater ability of the trees to recover in the latter type, though possibly the trees scattered through these hardwoods were not so severely defoliated. These figures, as do previous tabulations, show that the white spruce dying from defoliation was growing at a slower rate than the red spruce which died.

The Metis Lake plots (Tables X and XI) unfortunately (due to misunderstanding) were based on too few trees to make fair comparisons possible.

SUGGESTED APPLICATION

The recent widespread series of budworm epidemics in eastern Canada and northeastern United States, coming as they did at a time when the softwood supplies of these regions are becoming depleted and when considerable interest in putting the forests on a sustained-yield basis is being manifested, will no doubt stimulate more intensive forest practices. At all events, the budworm is an ever-present menace to the growing of spruce and fir in these regions and must be given due consideration in the application of any silvicultural systems.

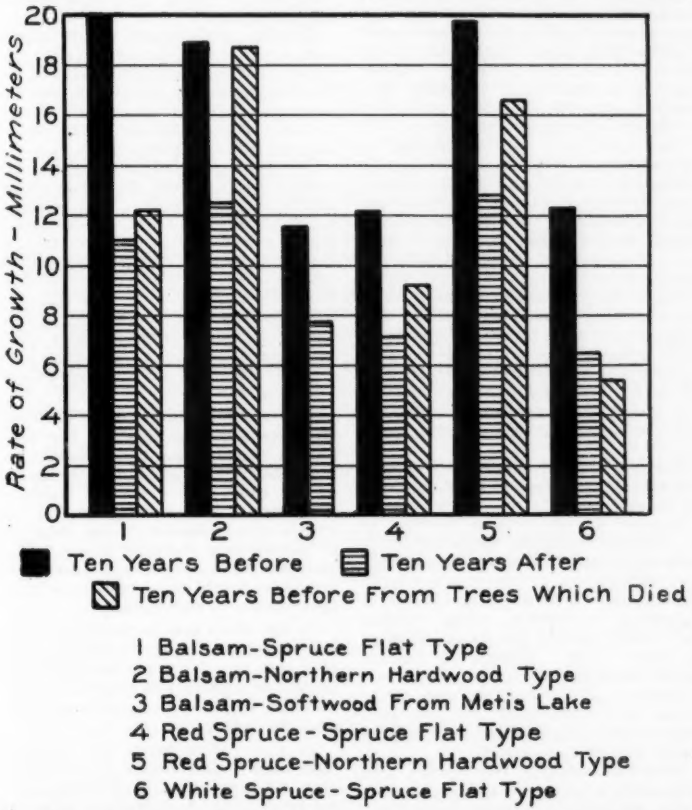

FIG. 5.—Diagram showing rate of growth of spruce and fir, occurring in various forest types, for the 10-year periods preceding and following spruce budworm defoliation

Our knowledge of how budworm outbreaks originate and the factors necessary for their phenomenal increase and spread is so meager that any recommendations for the prevention of future outbreaks are only speculative. When outbreaks do occur, we can hope for little relief from direct control measures, though prompt salvage of the defoliated material will greatly reduce the total losses.

With such limited possibilities of prevention or control before us, the only alternative is to keep the future forests in a condition least susceptible to the effects of defoliation. It is believed that the present study indicates that this can be accomplished by maintaining thrifty and vigorous stands.

This result can only be secured in natural stands by judicious cuttings to reduce the density and promote more rapid increment in the individual trees. As an example, in second-growth stands, such as those under consideration, varying from 20 to 50 cords per acre at 40 to 50 years of age, it would certainly be practical to make a pulpwood cutting when competition becomes so severe as to induce high mortality from defoliation. Such an operation should remove from 5 to 10 cords per acre, so as to make it profitable, and it should be conducted with the idea of maintaining trees of better quality.

The selection of the trees for thinning should be governed by the following considerations:

Remove all the balsam possible to the smallest possible diameter limit, since it is most susceptible to budworm defoliation, promotes heavy feeding, and may be an important factor in originating outbreaks. Any balsam that is left should be single, thrifty, dominant trees. Groups of balsam should never be left.

Remove all inferior red and white spruce and those that will not gain dominance by the time the next logging operation is contemplated.

Between doubtful red spruce and white spruce favor red spruce, since it better withstands adverse conditions and is a more persistent grower. The presence of red spruce is least effective in promoting severe budworm defoliation.

These thinnings should induce reproduction and, judging by the effect of bud-worm thinnings, this regeneration may be largely balsam. However, by removing practically all of the balsam and by breaking the soil litter through logging, possibly a high percentage of spruce can be secured.

If the stands are being managed for pulp wood, 10 to 20 years later, after reproduction is established, clear cutting should be adopted. In this case the new crop could again be mixed with balsam.

If saw material is desired later, pulpwood thinnings should be made to reduce density and promote more rapid growth, following the same selection as before but favoring red spruce still more because of its quality of persistent growth.

Balsam and white spruce should only be grown on the better sites. On these balsam is sufficiently immune to budworm feeding up to 40 years to leave a well-stocked stand, though several years' increment will be lost and the rotation lengthened.

In the spruce swamp type, where reproduction is very good and balsam practically negligible, some form of selection system is advocated which would aim to remove mature and less thrifty trees, giving room for younger trees which grow more rapidly. Further study is needed in this type to determine the causes of periodic cycles of rapid growth in older stands and the conditions favoring rapid growth observed in younger stands following fires.

Any recommendations in hardwood mixtures are dependent on the possibilities of utilization of the hardwoods. Since hardwoods are only a protection to the softwoods while the latter are overtopped, and since once the softwoods gain dominance the mortality of balsam from the budworm is as high or higher than in pure softwoods, even greater care will be necessary to handle these mixtures successfully.

In the birch and poplar type, which is a transitional stage in the formation of the spruce flat type, the hardwoods should be regarded as purely a shelter, and the earlier the conifers are liberated the better.

In the yellow birch and northern hardwood types, where the softwoods grow very rapidly and where practical conversion to a softwood type will probably be an impossibility, efforts should be made to utilize or dispose of as much of the hardwoods as possible to liberate higher proportions of softwoods.

In hardwood mixtures balsam is most susceptible as a free tree and white spruce as an overtopped tree, which demands early cutting of free balsam and early liberation of overtopped white spruce. The spruces, particularly white spruce, should al-

ways be favored in preference to balsam.

Mature softwood types were not studied in detail, though it is believed methods involving clear cutting with the object of securing more uniform second-growth stands in compartments of varying ages are more desirable. The history of these northern forests in the past has been largely the history of burns, the resulting second growth producing relatively more budworm-resistant forests, with lower percentages of balsam. There may be a lesson in this, suggesting periodic clear cutting, a case somewhat analogous to the better results secured by periodic renewal of coppice forest by seedling trees.

Diversified forests, both as to age classes and types, will aid in lowering the momentum of budworm outbreaks and result in less general and disastrous devastation.

These recommendations are not to be considered as applicable to the Laurentian region. The entirely different silvicultural characteristics of the spruce of this region, the great difficulty of securing spruce reproduction, and the prolificness of balsam will demand different methods.

SUMMARY

The study herein reported indicates that there is a definite correlation between the mortality occurring in spruce and fir stands (from spruce budworm defoliation) and the rate of growth of these stands prior to attack. The more rapid the rate of growth as expressed in diameter increment the lower the resulting mortality under equal conditions of feeding. This relation between the effects of budworm feeding and previous vigor is a more tangible quantity than any of the other factors heretofore considered and should serve as a practical basis for preventive measures through proper silvicultural practices which maintain rapid growth.

A comparison of the rate of growth of trees surviving budworm attack shows that the diameter growth for the 10-year period following the first year of feeding is only about one-half that of the preceding 10 years.

It was found that in hardwood types the immunity of softwoods was proportional to the protection of the overstory of hardwood foliage. The percentage of mortality among dominant softwoods in mixed stands was as high as in pure softwood stands.

REVIEW OF THE NEMATODE GENERA SYNGAMUS SIEB. AND CYATHOSTOMA E. BLANCH.[1]

By EDWARD A. CHAPIN

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INTRODUCTION

Following the collection of a species of Cyathostoma from the red-tailed hawk (*Buteo borealis*) the writer has found it necessary to go into the question of the various described species of Syngamus and Cyathostoma in order to determine the status of the above-mentioned form. This investigation has shown that the species from the hawk is apparently new to science and in addition it has brought out several facts relating to the species of these two genera that seem to render desirable a general taxonomic review of the genera. The only previous paper of a similar scope is one in Russian by Skrjabin (1915), which is not readily available to most workers.

The collections of the U. S. National Museum contain abundant material for study of three species; H. A. Baylis, of the British Museum (Natural History), has kindly lent two pairs of a worm from an Old World corvine bird, and Herbert Fox, of the Zoölogical Society of Philadelphia, through F. D. Weidman, has submitted for study a series of specimens from three avian hosts. Thanks are extended for this coöperation and to B. H. Ransom for his helpful criticism.

The differential characters of value in the diagnosis of species of these genera have not as yet been well formulated and the separation of species on the basis of existing descriptions is in some cases difficult or impossible. Leiper (1913) in a paper on *Syngamus kingi* has made a useful contribution toward the formulation of characters that may be employed in the recognition of species. Though his paper has to do with the genus Syngamus only, some of his differentials may be applied to Cyathostoma equally well. He suggests as diagnostic or differential characters the following: (1) Relative position of buccal capsules in paired specimens; (2) relative length of esophagus to that of the body; (3) size and armature of mouth capsule; (4) relation of axis of buccal capsule to that of body; (5) outline of optical section of chitinous wall of capsule; (6) size of spicules; (7) configuration of posterior end of body of female; (8) site of excretory pore.

The first of these differentials of course can be used for only the species of Syngamus and then must be used with discretion. In young specimens of *S. trachea* the capsule of the male may be as much as half the length of the male in advance of the capsule of the female. On the other hand, there are before the writer gravid specimens of this worm from the turkey in which the capsule of the male is only slightly in advance of that of the female. This variation is due to the fact that the male changes little in length after maturity is reached, while the developing eggs cause apparent growth in length of the female. The second character mentioned by Leiper is apparently valid so far as the males are concerned, but is influenced in the case of the females by the factor mentioned above. The other differentials noted by Leiper appear to hold for both sexes at any age, with the possible exception of the last. The excretory pore is subject to a slight apparent migration with the egg development.

On the other hand, Leiper does not consider the bursa of the male. In the species of Cyathostoma there is no trouble involved in an examination of this organ. In Syngamus, with reasonable care, a preparation of the bursa of the male can be made without serious damage to the female worm. If with a sharp knife the genital cone of the female worm is removed from the body, the male worm with bursa entire comes away at the same time. A few minutes' careful work with a needle suffices to remove from within the bursa the fragments of the female genital cone, and the male may be then treated as any strongyle. If the pair of worms has been carried into glycerine before this operation is at-

[1] Received for publication June 24, 1924, issued June, 1925.

tempted, there is no extrusion or displacement of the female organs.

The following specific names have been applied to members of the two genera but are discarded. The reason for discarding each name is noted.

Syngamus bovis Willach 1896e. Railliet has stated (1897a) that this "species" is based on an artifact and not on a worm.

Syngamus coelebs Schlotthauber 1860a. This is a nomen nudum, based on certain worms from *Falco lagopus*.

Syngamus laryncheus Smit 1922. Lapsus calami for *S. laryngeus* Raill.

Syngamus major Smit 1922. Not distinct from *S. laryngeus* Raill.

Syngamus minor Smit 1922. *S laryngeus* Raill. renamed.

Syngamus mucronatus Schlotthauber 1860a. Nomen nudum, based on worms from *Picus canis* and *P. major*.

Strongylus pictus Creplin 1849a. *Syngamus trachealis* Sieb. renamed.

Syngamus primitivus Molin 1861a *Fasciola trachea* Mont. renamed.

Syngamus pugionatus Schlotthauber 1860a. Nomen nudum, based on worms from *Corvus pica* and *Sturnus vulgaris*.

Syngamus sclerostomum Molin 1861a. *Strongylus variegatus* Creplin renamed.

Sclerostomum syngamus Diesing 1851a. *Syngamus trachealis* Sieb. renamed.

Syngamus trachealis Sieb. 1836a. *Fasciola trachea* Mont. renamed.

There remain 11 apparently valid species which have been assigned to these genera. One at least of these, *Syngamus kingi* Leiper, appears to be out of its usual host and may be found to be synonymous with a previously described form. Another, *S. nasicola* Linst., may prove to be equal to *S. laryngeus* Raill.

SYNGAMUS VON SIEBOLD

Syngamus Sieb., 1836, Arch. Naturg., (Jahrg. 2) 1: 105–116

Generic characters. — Strongylidae; sexes permanently joined in copula; buccal capsules of both sexes large, heavily walled, furnished at the base with eight or nine teeth arranged about the center, the teeth of two distinct sizes. Excretory pore anterior to the esophago-intestinal junction; esophagus moderate, pestle-shaped. Males with thick-walled bursa; bursal rays short and thick; spicules small to very small (150 μ to 25 μ). Vulva of female in the anterior third of the body length; tip of female tail blunt or acute. Eggs moderate in size, operculated after deposition.

Habitat.—In the respiratory tract of birds and mammals.

Type species.—(*Syngamus trachealis* v. Sieb.) = *Fasciola trachea* Mont.

Six valid species which may be referred to this genus are recognized in this paper. The appended key emphasizes the points of difference. Two other species are considered doubtful and are treated at the end of the list.

KEY TO SYNGAMUS

1. Species infesting mammals 2
   Species infesting birds 3
2. Ovarian-uterine complex reaching just beyond the middle of the length; spicules 25 μ long, host *Bos taurus* ..... *S. laryngeus*.
   Ovarian-uterine complex reaching nearly to anus ..... *S. dispar*.
3. Branches of dorsal ray simple, not subdivided 4
   Branches of dorsal ray divided in apical portion 5
4. Spicules subequal, 50 μ long, host *Nucifraga caryocatactes* ..... *S. parvus*.
   Spicules distinctly unequal, right spicule 79 μ, left spicule 70 μ long, host *Corvus brachyrhynchos* ..... *S. gracilis*.
5. Each branch of dorsal ray bifurcate, spicules 150 μ long, host *Phalacrocorax carbo* ..... *S. microspiculum*.
   Each branch of dorsal ray trifurcate, spicules 60 μ long, hosts gallinaceous birds ..... *S. trachea*.

SYNGAMUS LARYNGEUS Railliet (pl. 1, fig. 4; pl. 2, figs. 12, 13, 16; pl. 4, fig. 44).

Syngamus laryngeus Raill, 1899, Compt. Rend. Soc. Biol. [Paris] (XI) 1: 18–21; Sheather and Shilston, 1920, Pusa [India] Agr. Research Bul. 92, 8 p.

S. laryncheus Smit, 1922, Deut. Tierärztl. Wchnschr. 30: 506–507. (Lapsus for *laryngeus*.)

S. Laryncheus minor Smit, 1922, Deut. Tierärztl. Wchnschr. 30: 507. (*laryngeus* Raill. renamed.)

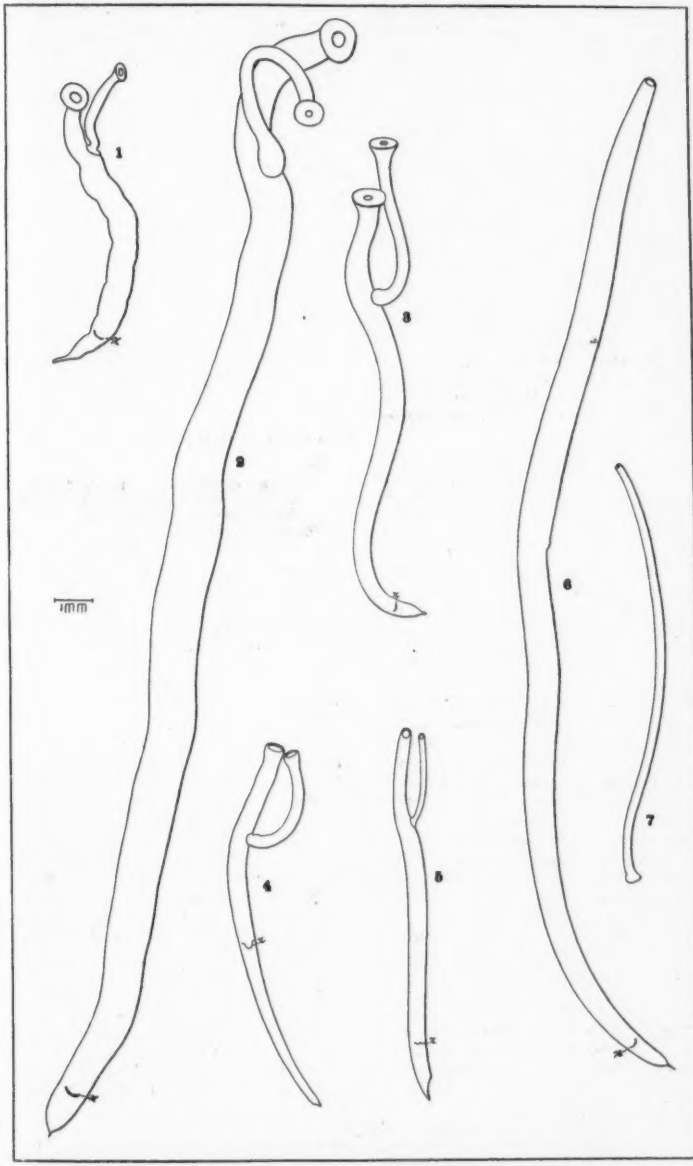
S. major Smit 922, Deut. Tierärztl. Wchnschr. 30: 507.

The material representing this species which is available for study consists of four lots, all from *Bos taurus*, as follows: One pair from Cochin China, received from A. Railliet in 1899; many pairs from Manila, Philippine Islands, received from William Boynton in 1914, and two lots from Rio Piedras, Porto Rico, received from J. Bagué in 1917. The specimens, a pair in copula, from Railliet may be considered in the light of type material.

Smit described spicules reaching from the end of the esophagus backward, presumably to the bursa. It is uncertain just what organ has been mistaken for spicules; there is, however, no doubt that the organs described are not spicules.

Six pairs have been examined in detail, one from Cochin China, three from Manila, and two from Porto Rico. There appears to be considerable variation in the actual sizes of the various organs, which, however, are not coordinated with the geographical distribution.

MALE.—The total length is from 2.6 to 3.6 mm. Sheather and Shilston report males as long as 5 mm. The buccal capsule in the Cochin China specimen is 0.23 by 0.27 mm.; in the others it is larger, attaining the size of



1. *Syngamus parvus* n. sp. Outline of pair (female gravid).
2. *Syngamus trachea* (Mont.). Outline of pair (female gravid).
3. Idem. Outline of pair (female immature).
4. *Syngamus laryngeus* Raill Outline of pair (female gravid)
5. *Syngamus dispar* (Molin). Outline of pair. (After Diesing.)
6. *Cyathostoma americana* n. sp. Outline of female (gravid).
7. Idem. Outline of male.

(x=posterior extent of uterine coils)

0.30 by 0.36 mm. in one from Manila. The esophagus is from 0.66 to 0.89 mm. long and from 0.7 to 0.25 mm. wide at the point of greatest width (cardiac dilation). The bursa is nearly circular. On the ventral line there is a notch which is more or less completely closed by a small lobe. The supporting rays may be distinguished only under extremely favorable condition and the form of their terminations has not been made out. From examination of several specimens it is evident that there is a complete set of rays; the dorsal, apparently undivided with the externo-dorsal rays are grouped closely together. The postero-lateral, medio-lateral, and externo-lateral rays form a compact group at the extremity of the transverse diameter. The ventro-ventral ray is close to the insertion of the ventral lobe, while the latero-ventral ray is just beside it.

In two specimens, which after dissection were in suitable condition for study, spicules were found. The small size of the spicules explains the failure of other observers to find them. They are similar in shape, about 25 μ long, and lie just inside the cloacal opening. The opening itself appears to be supported by a chitinous ring, perhaps a modified gubernaculum. Except for the ring, no structure was seen that could be identified as a gubernaculum.

FEMALE.—The position of the vulva in respect to the total length of the worm is less variable toward maturity in this species than in *S. trachealis*. This is due to the fact that the uteri do not extend much farther behind the vulva than they do in front of it. Thus in gravid specimens the distortion due to the ripe eggs is nearly evenly balanced before and behind, and the ratio obtaining in mature but not gravid worms is maintained in the gravid specimens. In the specimens measured, the ratio of the distance between the anterior end and the vulva to the total length of the worm varies from 1: 4.64 to 1: 4.69. Owing to the anterior distribution of the uteri, the mature worms assume a distinctive tapering form, the posterior third being perceptibly thinner than the anterior two-thirds.

A very young pair (female 3.58 mm. in length) shows certain features of interest. The spicules are easily seen without dissection. The vagina is very short; the bifurcation of the uteri occurs within the area covered by the male bursa. The uteri make a short anterior loop and then proceed posteriorly to a point less than half the distance from the vulva to the tail. The ovaries occupy most of the space between this point and the posterior extremity. The vulva divides the total body length of the female as 3 : 5 or 1 : 1. 66

It appears that in this species the ovarian tissue degenerates after the eggs are discharged into the uteri, for in gravid specimens the ovaries are not perceptible.

Host.—Bos taurus, doubtfully from Homo.

Location.—Larynx.

Geographic distribution.—Indo-Malayan region, Porto Rico, Brazil?.

Travassos (1922) reports a case of *S. laryngeus* in man (Brazil). There is no evidence given to show whether he was dealing with this species or another.

SYNGAMUS DISPAR (Diesing) (pl. 1, fig. 5).

Sclerostoma dispar Dies. 1851, Syst. Helm., 2: 303; Dies., 1857, Denkschr. Akad. Wiss., Wien, Math.-naturw. cl., 13 (abt. 1): 16.
Syngamus dispar Molin., 1861, Mem. R. Inst. Veneto Sci., Litt. ed Arti 9:565.

There is unfortunately no material of this species available nor is there a good or even a moderately good description extant. The available figures (Diesing) cover one or two important points not mentioned in the description. The following is a summary of the specific characters, taken from all available sources:

Mouth opening terminal; buccal capsule with ribs, presumably ending at the base in lancetlike teeth; bursa of male rather more strongly developed than in *S. laryngeus;* spicules probably minute (not described); vulva of female at about anterior fourth; coils of uterine complex reaching to posterior seventh of the total length; tail short, stoutly conical; anus just before tail. Length of male, 5–7 mm.; of female, 20–27 mm.

Host.—Felis concolor.

Location.—Trachea.

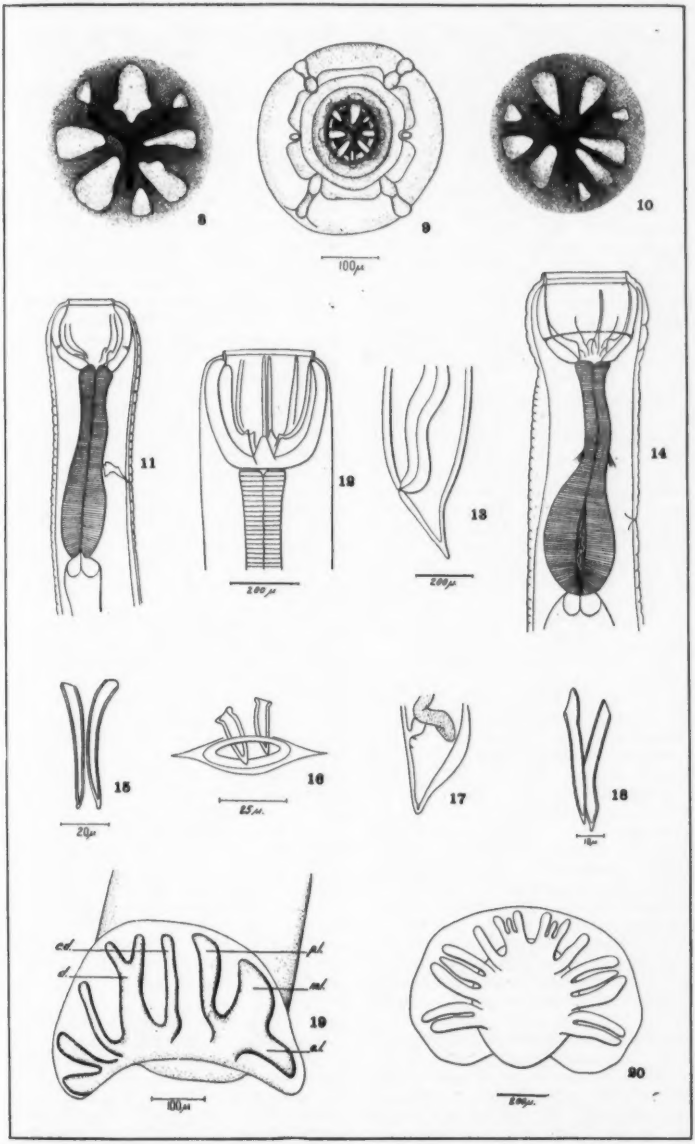
Geographic distribution.—Brazil.

Though little concerning this species is known, still it is highly probable that the species is valid. The only possible conflict is with *S. kingi* Leiper. A discussion of the relationship is included under that species.

Syngamus parvus n. sp. (pl. 1, fig. 1; pl. 2, fig. 15, 19).

Similar in general shape to *S. trachea* but much smaller and with other male secondary sexual characters.

MALE.—About 2.4 mm. long, 0.22 mm. in diameter, cylindrical, slightly



8. *Syngamus trachea* (Mont.) Arrangement of teeth in eight-toothed (normal) form.
9. Idem. Front view of head.
10. Ibidem. Arrangement of teeth in nine-toothed form.
11. *Syngamus kingi* Leiper. Side view of anterior end of male. (After Leiper.)
12. *Syngamus laryngeus* Raill. Side view of anterior end of male.
13. Idem. Side view of tail of female.
14. *Syngamus kingi* Leiper. Side view of anterior end of female. (After Leiper.)
15. *Syngamus parvus* n. sp. Spicules.
16. *Syngamus laryngeus* Raill. Spicules.
17. *Syngamus kingi* Leiper. Side view of tail of female. (After Leiper.)
18. *Syngamus trachea* (Mont.). Spicules.
19. *Syngamus parvus* n. sp. Dorsal portion of bursa of male.
20. *Syngamus trachea* (Mont.). Arrangement of rays in bursa of male

constricted in neck region. Buccal capsule heavily chitinized, 178 μ in depth, 207 μ in its greatest inside diameter, walls 29 μ thick. Buccal teeth and circumoral papillae as in *S. trachea*. Esophagus about 326 μ in length. Nerve ring? Excretory pore? Bursa 350 μ in diameter; ventral rays short, stout, and approximate; lateral rays stout; medio-lateral ray much so; externo-lateral ray arising from the side of the medio-lateral; postero-lateral ray slender in comparison with the other two. Externo-dorsal ray more slender and parallel than postero-lateral; dorsal trunk bifurcated near tip, each bifurcation simple. Spicules short, about 49 μ, similar in shape to those of *S. trachea*.

FEMALE.—About 7.8 mm. long, 0.65 mm. in diameter at widest part. Neck region just back of buccal capsule about 0.35 mm. in diameter. Buccal capsule 300 μ in depth, 440 μ in average diameter. Buccal teeth as in *S. trachea*. Nerve ring? Excretory pore? Esophagus short and thick. Vulva in gravid worm dividing total length into ratio as 1:5.2; uterine coils extending backward to 1.25 mm. from apex; anus subterminal; tip of tail very blunt. Eggs 74 by 44 μ, not yet segmented in uterus.

Host.—*Nucifraga caryocatactes*.

Habitat.—Trachea.

Geographical distribution.—Not stated for present specimens. (Host occurs in Europe.)

Syntypes.—British Museum (Natural History), part of lot No. 92.11.4.1–3. One pair deposited in U. S. N. M. Helm. Coll., No. 26098.

Through the kindness of H. A. Baylis, the author has had the opportunity to study the worms upon which the foregoing description is based. Unfortunately, the preservation of the specimens is not of the best, but it is believed that sufficient differences can be made out to warrant the setting apart of the material from Nucifraga as a species distinct from *S. trachea* (Mont.). Thanks are extended to Baylis for securing the gift of one pair for the National Museum collection.

Both of the species of Syngamus described in this paper from corvine hosts agree in having the dorsal ray of the male bursa branched, the branches simple. In the present species the branching occurs beyond the middle of the length. In the American form, *S. gracilis*, the bifurcation is basal, resulting in a total suppression of the common dorsal trunk.

Syngamus gracilis n. sp. (pl. 4, figs. 38, 40, 42).

Syngamus trachealis Wied., (pars), Fox, 1923, Disease in captive wild mammals and birds p. 650.

Superficially resembling *S. trachea* (Mont.), but smaller and more slender. Male from 3 to 3.3 mm. in length, cylindrical, thickness 270 μ. Buccal capsule heavy, its wall 45 μ thick; the diameter of the lumen of the capsule near the upper edge is about 200 μ, the depth 165 μ. Buccal teeth as in *S. trachea;* the largest have an altitude of 45 μ. The esophagus is nearly 600 μ long, clavate, its greatest diameter 135 μ. Nerve ring, excretory pore, and cervical papillae not seen.

Caudal bursa narrow and deep; branches of the dorsal ray 150 μ long; externo-dorsal ray slightly shorter, measuring 132 μ. The lateral rays are mutually contiguous, parallel, and about the size of either branch of the dorsal. The ventral rays are smaller and more slender. The spicules are distinctly unequal; the right is bent and measures 79 μ long, while the left is nearly straight and is but 69 μ in length.

The female is from 8 to 11 mm. long and is proportionately stouter than the male. The buccal capsule is relatively enormous, its internal dimensions being 525 μ wide by 300 μ deep. The wall is 50 μ thick. Teeth as in *S. trachea*. The esophagus is 825 μ long and 225 μ thick at the point of its greatest diameter, just before the esophago-intestinal valve. Other structures in the neck region not seen. The vulva is 1.4 mm. behind the anterior end or at about the anterior eighth of the body length. The vagina is longer than in related species, and both branches of the uterus proceed backward. The anus is about 300 μ in front of the very blunt posterior extremity. The eggs range when mature from 79 to 83 μ in length by 40 to 46 μ in width.

Host.—Corvus brachyrhynchos.

Location.—Trachea.

Geographic distribution.—Philadelphia Zoölogical Park, Nov. 14, 1914.

Type and allotype.—U. S. N. M Helm. Coll. No. 26127. Paratypes in collection of the Zoological Society of Philadelphia.

This species is more closely related to *S. parvus* from *Nucifraga caryocatactes* than to *S. trachea* of the gallinaceous birds. Yet it is in all probability the form which normally occurs in the

American crow. It is further probable that *S. trachea*, which can hardly sustain itself in a host as close phylogenetically to the turkey as the domestic fowl is, cannot under even the most favorable circumstances reach maturity and egg production in the crow. From this it seems probable that the crow is not, as often stated, a reservoir for the turkey and chicken gapeworm.

SYNGAMUS MICROSPICULUM Skrjabin (pl. 4, fig. 39).

Syngamus microspiculum Skrjabin, 1915, Vestnik Obshch. Vet. 27: 646.

Through the kindness of Robert J. Formad, the writer is able to offer the following translation of Skrjabin's original description:

MALE.—Cylindrical in shape, dark brownish in color, 3.9 to 4 mm. in length. In the region of the posterior portion of the esophagus the body is 0.29 mm. in diameter, while just before the bursa the diameter is 0.27 mm. The mouth capsule is 0.17 mm. deep by 0.22 mm. broad and is provided within at the base with three pointed triangular teeth, each 0.07 mm. long. The esophagus is 0.58 mm. long and is flask-shaped at its posterior end. The bursa of the male is very characteristic; the dorsal ray is dichotomously divided, the externo and postero-lateral rays form a compact group, and the latero-ventral and ventro-ventral rays are approximate. In this species the spicules are unusually characteristic. They are two in number, of similar size, and their length measures 0.15 mm.[2] As will be seen later, no one species of Syngamus has such small spicules, and for this reason this organ serves as the principal diagnostic sign of the species. The spicules have a thickened base and taper toward the posterior end; their color is brownish.

FEMALE.—Length 11 mm.; diameter in the region of the posterior portion of the intestine 420 μ, in the region of the vulva 500 μ, and near the anus 250 μ. The buccal capsule is broader than deep, its diameter being 250 μ and its depth 340 μ. The clavate or bottle-shaped esophagus is 765 μ long. The vulva is situated 4.45 mm. from the anterior end. The eggs are 75 μ long by 48 μ in transverse diameter. Their shape is very characteristic. One side of the egg is flat, the other hemispherical. The tail of the worm is acutely conical, similar to that of *S. variegatus* Crepl.

Syngamus microspiculum is distinguished from all described species by the following: (1) Size of spicules; (2) there are three teeth in the buccal capsule; (3) the position of the vulva; (4) the shape of the eggs.

Host.—*Phalacrocorax carbo.*

Location.—Trachea.

Geographic distribution.—Turkestan.

Skrjabin's description of three buccal teeth would seem to demand confirmation. Furthermore, the statement that the spicules of this form are the shortest known in the genus is not accurate. At the time Skrjabin's paper was written the spicules of *S. trachea* were already described as 60 μ in length. The dorsal ray of the bursa and the spicules are, however, ample to differentiate this species from any other known.

SYNGAMUS TRACHEA (Montagu) (pl. 1, fig. 2; pl. 2, figs. 8 to 10, 18, 20).

Wissent., 1799, Med. & Phys. Jour. [London 2: 204–205.

Fasciola trachea Mont., 1811, Mem. Werner. Nat. Hist. Soc. Edinb., (1808–10) 1: 176–193.

Syngamus trachealis, Sieb., 1836, Arch. Naturg. (Jahrg. 2), 1 (1): 105–116; Ransom, 1921, U. S. Dept. Agr. Bul. 939, 13 p.; Ortlepp, Jour. Helminthology, 1: 119–140.

Syngamus trachea (Mont.) Chapin, 1924, present paper.

In accordance with the International Code of Zoological Nomenclature, the name of this species should be *trachea* rather than *trachealis*, the former having some years' priority.

Much stress has been laid in the past on the position of the vulva as a taxonomic character. When used with caution and with due consideration for the age of the specimen, it is of considerable value. However, it must be recognized that as the worm ages and the uteri become packed with eggs there is a stretching, not only in the diameter of the worm but in the length. For instance, in the present species the vulva is well forward and the uterine complex is largely behind it. In young worms, quite immature so far as egg production is concerned, the ratio of the distance from the anterior end to the vulva to the total length of the worm is 1 to 3.25. In specimens showing a few eggs the ratio changes to 1 : 4.25, while in gravid specimens the ratio may become as

[2] In a later paper (Ann. Mus. Zool. Acad. Imp. Sci., Petrograd (1915) 1916, 20: 467–471), Skrjabin has redescribed this species and has given notes on certain other species of the genera considered here. The noteworthy difference between the two descriptions is that the spicules are described first as 150 μ long, later as 115 μ. Either statement may be true but it is impossible to state which was the intended figure by a study of the context. Also in the later paper the length of the spicules of *Syngamus trachealis* (= *S. trachea*) is given as 0.69 mm., a figure about ten times greater than that usually accepted. This is undoubtedly a typographical error for 69 μ.

high as 1 : 5. That the variation here noted is due to the presence of large numbers of eggs is shown by a corresponding study of *S. laryngeus* Raill., where the uterine complex extends hardly farther behind the vulva than it does in front of it.

Leiper has placed considerable emphasis on the relation of the axis of the buccal capsule to that of the body of the worm, stating that the capsule is turned dorsally. In old pairs it is true that the mouth opening is directed dorsally, but in young worms the mouth is directed anteriorly. The capsule is at all times terminal; any changes in the relative directions of the axes is due to a flexure of the neck region.

The teeth at the base of the buccal capsule have heretofore been described as eight in number but with no statement as to the relative sizes of the teeth. The normal form shows eight teeth, as follows: A large medium dorsal tooth which may or may not show two cusps. On either side of this tooth there is a very small submedian dorsal tooth. There are two large (always unicuspid) lateral teeth, one on each side, and two large submedian ventral teeth, each adjacent and similar to a lateral tooth. The ventral tooth is the smallest of all.

Occasionally there are nine instead of eight teeth, in which case the median dorsal is divided to form two teeth.

The spicules of ten males selected at random from material taken from both turkey and fowl vary in length from 57 μ to 64 μ. It is probable that reports of 140 μ spicules in this species refer to another species.

Host.—*Meleagris gallopavo, Gallus domesticus.*

Location.—Trachea.

Geographic distribution.—North and South America, Europe, Africa, Australia.

As Ransom has shown, the turkey should be considered the true host of this parasite. Chickens are readily susceptible to infection only when very young, and infection usually proves fatal. If the chicken survives the infection it loses its parasites within a short time, so that there is little opportunity for spread of infection from chickens. Available evidence indicates that in the absence of turkeys gapeworm infection is rare among chickens. Under these circumstances it would seem desirable to compare more carefully than appears to have been done specimens of gapeworms that have been collected from various species of birds other than the Galliformes to determine whether they are really *Syngamus trachea.*

SYNGAMUS KINGI Leiper (pl. 2, fig. 11, 14, 17).

Syngamus kingi Leiper, 1913, Trans. Soc. Trop. Med. & Hyg. [London] (1912–13), 6: 265–297.

The material on which this species is based was collected from *Homo sapiens* in St. Lucia, West Indies. From Leiper's description one may say definitely that the species is not *S. laryngeus* Raill., since the coils of the uterine complex reach to just in front of the anus; nor can it be *S. trachea*, for in that case the opening of the mouth capsule is turned dorsally instead of being terminal. The possibility that *S. dispar* and *S. kingi* are synonymous is not excluded. As has been pointed out, the transverse level of the month capsules in all species I have examined varies according to the age of the specimens, and the differentiation between *S. kingi* and *S. dispar* made by Leiper on the basis of this character accordingly seems open to question.

SYNGAMUS NASICOLA Linst.

Syngamus nasicola Linst. 1899, Mitt. Zool. Samml. Mus. Naturk. 1 (2): 18.

MALE.—5.6 mm. in length, 0.47 mm. in thickness. Buccal capsule 320 μ deep by 480 μ in breadth, with six ribs proceeding from the teeth on the internal wall. Esophagus ten forty-sixths of the body length.

FEMALE.—20.6 mm. long, 0.87 mm. thick. Esophagus one fifty-first (sic.) of the body length; tail one fifty-second of the body length and acutely conical. Vulva divides body as 3: 10. Eggs 88 by 46 μ.

Host.—*Cervus rufus; Capra hircus.*

Habitat.—Posterior nares, nasal cavity.

Geographical distribution.—Brazil (Rio Grande do Sul), Africa (Cameroon).

In the foregoing description, which contains all of the pertinent points mentioned in the original, there is only one point that serves to differentiate this species from *Syngamus laryngeus.* That point is the proportional dimensions of the buccal capsule of the male. If von Linstow was correct in saying that the capsule measured was that of the male, the capsule of the female should be very shallow and the two species are probably distinct. On the other hand, the figures cited are approximately those of the female capsule of *S. laryngeus.* The other

characters mentioned apply to *S. laryngeus*, and for the present it seems best to consider von Linstow's species as doubtful. As the descriptions of *S. laryngeus* Raill. and *S. nasicola* Linst. appeared almost at the same time, and as von Linstow made no mention of Railliet's species, it is probable that he was unaware of the existence of *S. laryngeus* Raill. Certainly the case appears to be that of the simultaneous publication of the same species under two specific names.

CYATHOSTOMA E. BLANCH.

Cyathostoma E. Blanch. 1849, Ann. Sci. Nat., Zool. (III) 11: 182.

Generic characters.—Strongylidae, sexes not joined in permanent copula,; buccal capsules of both sexes large heavily walled, furnished at the base with six or seven teeth arranged about the center, the teeth of two distinct sizes. Excretory pore as in Syngamus. Esophagus as in Syngamus. Males with normal strongyliform bursa, rays slender and sometimes (dorsal ray) branched, spicules long (more than 400 μ) and filiform. Vulva of female variable in position, sometimes in anterior third, sometimes median or slightly postmedian; tip of female tail acute. Eggs moderate in size, operculated after deposition.

Habitat.—In respiratory tract of birds.

Type species.—*Cyathostoma lari* E. Blanch.

Seven species, apparently all valid, may be referred to the genus *Cyathostoma*. At present, however, it is necessary to rely on certain unsatisfactory characters in order to form the key to species which appears below.

1. Vulva at or near the anterior third of the body 2
   Vulva near the middle of the body 3
2. Eggs 90 μ by 60 μ; spicules 600 μ long; hosts Anseriform birds ... *bronchialis.*
   Eggs 80 μ by 55 μ; spicules 650 μ long; host *Casuarius galeatus* ... *boulardi.*
   Eggs 80 μ by 40 μ; spicules ? long; hosts Ciconiform birds ... *variegatum.*
   Eggs 56 μ long; spicules 500 μ long; host *Tadorna tadorna* ... *tadornae.*
3. Male unknown; female small, up to 13 mm. long; in orbital cavity of *Larus ridibundus* ... *lari.*
   Male known, female larger ... 4
4. Spicules of male 490 μ long, gubernaculum 67 μ long; female up to 30 mm. long; in thoracic air sac of *Buteo borealis* ... *americanum*
   Spicules of male 660 μ to 720 μ long, gubernaculum 92 μ; female up to about 20 mm. long; in trachea of *Coscoroba coscoroba* ... *coscorobae.*

CYATHOSTOMA BRONCHIALIS (Muehlig) (pl. 3, fig. 32 to 34.)

Syngamus bronchialis Muehlig, 1884, Deut. Ztschr. Thiermed., 10: 265; Raill., 1898, Compt. Rend. Soc. Biol. [Paris] (X) 5: 400.
Cyathostoma bronchialis (Muehlig) Chapin, 1925, present paper.

MALE from 4 to 5.8 mm. in length, 260 μ in diameter, slightly attenuate anteriorly. Esophagus about 350 μ long. Caudal bursa entire; main trunk of the dorsal ray longer than the branches, which diverge sharply and are irregular in outline. Externo-dorsal and externo-lateral rays short, simple, subequal, each slightly reflexed at tip. Lateral rays arising from a common trunk, medio and postero-lateral rays separate, their tips approximate. Ventral rays separate and equal, the rays about as long as the common trunk; spicules thin, 510 to 620 μ long; internal margin fimbriate; basal portion feebly dilated; apices incurved and pointed.

FEMALE from 16 to 31 mm. long, 700 to 900 μ in diameter. Buccal capsule 325 μ broad by 205 μ deep. Esophagus very variable in length, according to age of worm, from one-thirteenth to one-thirtieth of the total length. Tail short, conical, sometimes curved. Anus 160–300 μ before the extreme apex. Vulva slightly prominent, just in front of the anterior third. Eggs narrowly ovoid, 74 to 83 μ long by 49 to 62 μ wide, with an inconspicuous operculum at the narrow pole.

Hosts.—Domestic geese, swan.

Location.—Trachea and bronchi.

Geographic distribution.—Europe and North America.

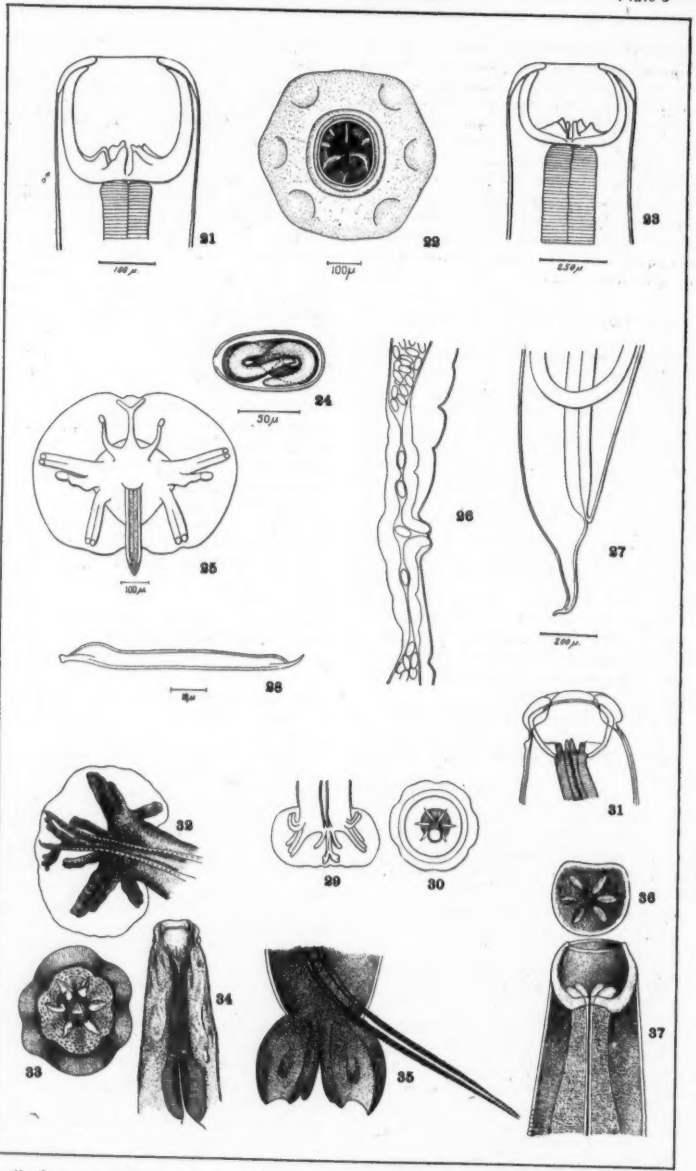
There is a single female in the collection from a swan, sent from Ithaca, N. Y., by W. A. Hagan, April, 1920. Although not in the best condition for study, it does not appear to be different from the above.

CYATHOSTOMA BOULARTI (Mégnin) (pl. 3, fig. 35 to 37).

Sclerostoma boularti Mégnin, 1884, Jour. Anat. et Physiol., 20–457.
Syngamus boularti (Mégnin) Raill., Compt. Rend. Soc. Biol. [Paris] (X) 5 : 402.
Cyathostoma boularti (Mégnin) Chapin, 1925, present paper.

Body soft, colored bright red by intervisceral fluid; intestine spiral, much longer than the body; head narrower than the neck.

MALE 7 mm. long, 450 μ thick. Head narrow, 200 μ in diameter; buccal opening terminal, 120 μ in diameter. Body narrowly cylindrical, truncate



21. *Cyathostoma americanum* n. sp. Side view of anterior end of male.
22. Idem. Front view of head.
23. Ibidem. Side view of anterior end of female.
24. Ibidem. Egg from culture (88 days old).
25. Ibidem. Bursa of male.
26. Ibidem. Female genitalia in the region of vulva.
27. Ibidem. Side view of tail of female.
28. Ibidem. Gubernaculum.
29. *Cyathostoma variegatum* (Crep.) Bursa of male. (After Linstow.)
30. Idem. Front view of head. (After Linstow.
31. Ibidem. Side view of head. (After Linstow.)
32. *Cyathostoma bronchialis* (Muehlig). Bursa o male. (After Muehlig.)
33. Idem. Front view of head. (After Muehlig.)
34. Ibidem. Side view of head. (After Muehlig.)
35. *Cyathostoma boularti* (Mégnin). Ventral portion of bursa of male. (After Mégnin.)
36. Idem. Front view of head. (After Mégnin.)
37. Ibidem. Side view of head. (After Mégnin.)

posteriorly, terminated by a 2-lobed membranous bursa, each lobe of which is supported by five rays; spicules two, thin, each 650 μ long.

FEMALE from 18 to 20 mm. long, diameter 850 μ. Head 500 μ in diameter, with buccal opening 350 μ across. Body cylindrical, fish-hook shaped, with a short conical tail. Anal opening just before the posterior extremity; vulva prominent, situated at the anterior third of the body length. Oviparous. Eggs smooth, ovoid, 80 by 55 μ, with an opercle at the smaller end.

Host.—Casuarius galeatus.

Location.—Trachea.

Geographic distribution.—Australia. (Original lot from host in the Zoölogical Garden, Paris.)

The above is a free translation of the original description by Mégnin. No specimens are available for examination. The original figure of the bursa does not at first glance seem plausible. In mounting a specimen of *C. americanum* for examination the present writer happened to secure an almost identical picture. I believe it may be safely assumed that Mégnin failed to figure the dorsal half of the bursa which was superimposed on the worm. The portion figured shows the ventral rays centrally located, the medio and postero-lateral rays together as the lateral fields in Mégnin's figure. The apices of the externo-lateral rays appear in the figure as the isolated oval spaces, one in each lobe. From the length of the spicules, and from the host, it is, I believe, wise to consider the species as valid.

CYATHOSTOMA VARIEGATUM (Creplin) Emend. (pl. 3, fig. 29 to 31).

Strongylus trachealis Nathusius, 1837, Arch. Naturg. (Jahrg. 3) 1: 60. (nec. *Syngamus trachealis* Sieb. 1836.)
Strongylus variegatus Creplin, 1849, Arch. Naturg. (Jahrg. 15) 1: 64. (*Strongylus trachealis* Nath. renamed.)
Syngamus variegatus (Creplin) Raill. 1898, Compt. Rend. Soc. Biol. [Paris] (X) 10: 401.
Cyathostoma variegatum (Creplin) Chapin, 1925, present paper.

MALE 7.8 mm. to 9 mm.; female 13.5 mm. to 45 mm. in length. Body slightly attenuated in either direction; posterior extremity of female ending in an acute tail, that of male truncate. Mouth opening circular, very broad, leading into a buccal capsule with heavy chitinous walls. Buccal teeth six in number (?), corresponding in position with the circumoral papillae. Esophagus flask-shaped, with a long neck; in the male the esophagus is more slender. In the female the anus opens just before the tip of the tail; in the male it is within the bursa. Vulva prominent, transverse, crescentic, situated at the interior third of the body length. Eggs 80 μ by 40 μ. Bursa of male strongyliform, dorsal ray bifurcate. Spicules long, mutually similar, filiform.

Host.—Ciconia nigra.

Location.—In trachea.

The above-mentioned data are taken from the original description of Nathusius and are sufficient merely to show that the species is one of the four known forms in which the vulva lies at the anterior third. A knowledge of the exact length of the spicules would probably serve to determine the validity of one or more of the other species.

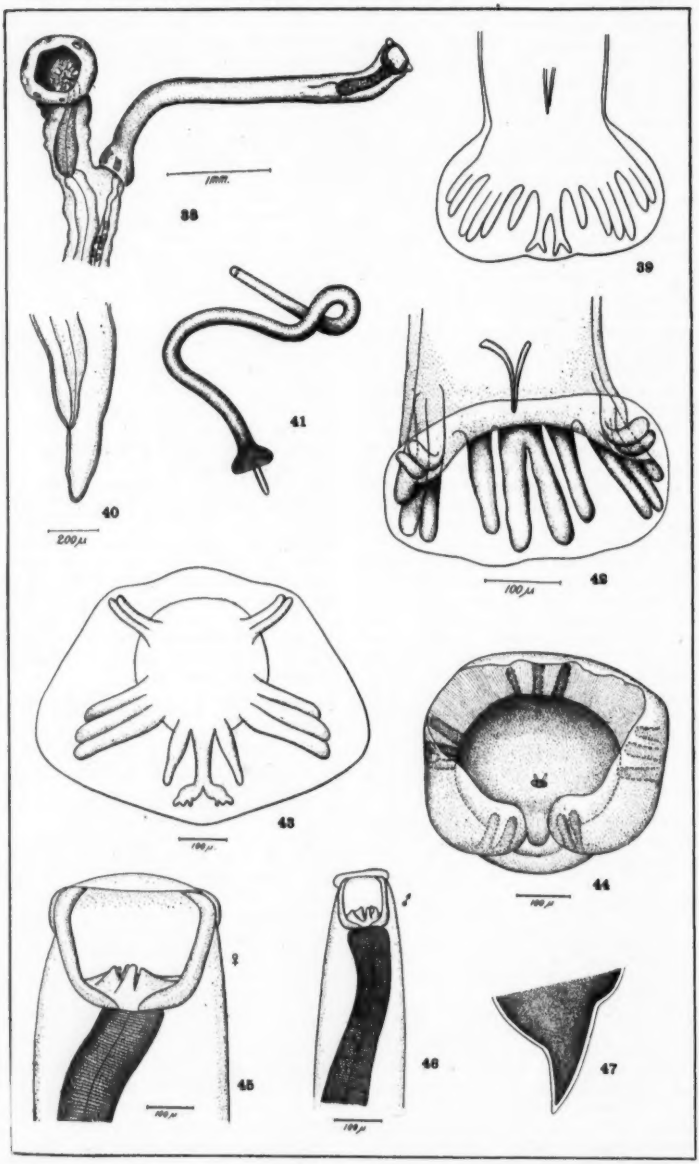
At first glance it might seem that a combination of *Cyathostoma* with *trachealis* Nathusius would be the correct name for this species, since there is no conflict with *trachealis* Sieb. Nathusius did not intend to name any species. He misidentified the material before him, and on the strength of his identification he transferred Siebold's species from Syngamus to Strongylus. While the International Code of Zoölogical Nomenclature is not explicit on the point, it would appear that for the sake of stability of nomenclature an unintentional creation of a name should be disregarded. A misidentification is not the proposal of a new name nor can it be regarded as tantamount to such.

CYATHOSTOMA TADORNAE Chatin (pl. 4; figs. 41, 47).

Cyathostoma tadornæ Chatin, 1874, Ann. Sci. Nat. Paris (VI) 1 (Art. 6): 1–6.
Sclerostoma tadornæ Linst., 1878, Compend. Helm, p. 152.
Syngamus tadornæ Raill., 1898, Compt. Rend. Soc. Biol. [Paris] (X) 5 : 402.

MALE.—Length 9.6 mm., form cylindrical, of uniform thickness throughout; color brick-red. Cuticle transversely striate at intervals of 20 μ, longitudinal striae apparently absent. Buccal capsule corneous and cup-shaped, furnished at its base with two pharyngeal teeth. Esophagus thick, clavate, about one-fifteenth of the body length. Body is terminated by a bursa, well developed and regular, which is supported by ten rays, four of which are simple, the remaining six being bifurcate at their apices. From within the bursa there extend two brown spicules, 500 μ long.

FEMALE.—Length 23 mm. Body of a brighter red than that of male. Head 0.9 mm. in diameter, similar to that of male. Buccal capsule as in male. Esophagus 1.8 mm. long, clavate. Vulva situated near the anterior third of the body. Uteri well developed, packed with eggs measuring in their longest diameter 0.56 mm. (sic!). Body terminated by a conical



38. *Syngamus gracilis* n. sp. Male and anterior portion of female.
39. *Syngamus microspiculum* Skrj. Bursa of male. (After Skrjabin.)
40. *Syngamus gracilis* n. sp. Posterior extremity of female.
41. *Cyathostoma tadornae* Chatin. Male, entire worm. (After Chatin.)
42. *Syngamus gracilis* n. sp. Bursa of male.
43. *Cyathostoma coscorobae* n. sp. Bursa of male.
44. *Syngamus laryngeus* Raill. Bursa of male.
45. *Cyathostoma coscorobae* n. sp. Buccal capsule of female.
46. Idem. Buccal capsule of male.
47. *Cyathostoma tadornae* Chatin. Posterior extremity of female. (After Chatin.)

process, set at an angle with the longitudinal axis of the body.

Habitat.—In the trachea of *Tadorna tadorna* L.

There is no evidence to show that the buccal capsule of this species was examined otherwise than from the side, and for that reason the statement concerning the number of buccal teeth may be questioned. There are probably six or seven teeth present, as in the other species of the genus. The size given for the egg is ten times too large, according to the figure. The magnification as given on the plate gives a length of about 60 μ.

CYATHOSTOMA LARI E. Blanchard.

Strongylus sp., Siebold, 1837, Arch. Naturg (Jahrg. 3) 1: 68.
Cyathostoma lari E. Blanch., 1849, Ann. Sci. Nat. Zool. (III) 11: 183.
Sclerostoma cyathostomum Dies., 1851, Syst. Helm., 2: 306. (*Cyathostoma lari* E. Blanch. renamed.)
Sclerostoma lari (Blanch.), Molin, 1861, Mem. R. Inst. Veneto Sci. Lett. ed. Arti, Venezia, (1860) 9: 561.
Syngamus lari (Blanch.), Ransom, 1904. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 60: 43, figs. 38–40; Linst., 1909, Süsswasserf. Deutschl., Heft 15, Parasit. Nematoden, p. 62.

Body red in color, attenuated anteriorly. Mouth opens into a pharyngeal capsule, which is marked in front by an annulation. Esophagus is thick and muscular, and gradually increases in diameter posteriorly. Intestine rather sinuous, with thin, delicate walls, dark in color.

MALE 6 to 8 mm. long (according to Siebold). Body ends abruptly behind; membranous fanlike bursa with six rays, of which the middle rays are the most marked; two spicules of equal length. Testicle a single very wide tube, sinuous anteriorly.

FEMALE 6 to 13 mm. long by 0.5 to 11 mm. thick. Body becomes gradually thicker in the anterior third, then continues of equal thickness to the posterior extremity. Tail with slender conical tip. Cuticle finely striate. Esophagus equal to one-fifteenth of the body length. Ovaries two, widening into capacious uteri, which unite to form a vagina of about 2 mm. in length. The latter gradually decreases in diameter toward the vulva, a large, prominent opening, with salient lips, situated slightly posterior to the middle of the body.

Habitat.—In the nasal and orbital cavities of *Larus ridibundus*, *Larus fuscus*, and *Larus* sp.

The above description is taken from Ransom's paper and is a compilation of all the available data. It is unfortunate that so little is known of the type species of this genus.

Cyathostoma americana n. sp. (pl. 1, fig. 6, 7; pl. 3, fig. 21, 28).

MALE 12 mm. long, cylindrical. anterior fifth somewhat attenuate; Buccal capsule about as wide as deep, internal transverse diameter 185 μ; depth of the chitinous portion 170 μ; wall of capsule 25 μ thick. Buccal teeth six or seven in number, subacutely triangular in shape, occupying the entire floor of the capsule; teeth not continued up the sides of the capsule in the form of ridges. The largest (lateral) teeth are about 57 μ high. Head papillae six in number, all essentially similar, arranged in a circle, the plane of which lies 60 μ behind the anterior extremity. Each papilla appears as a rounded knob, 10 μ in diameter. Esophagus 730 μ long, clavate, 74 μ thick at the anterior end, 130 μ thick just before the esophago-intestinal valve, nerve ring at the middle of the length, cervical papilla at about posterior seventh of esophagus, excretory pore just before the beginning of the intestine. Entire cuticle of worm smooth, without transverse striations.

Caudal bursa well developed, when spread 750 μ in transverse diameter; ventral rays similar and approximate; externo-lateral shorter than other lateral rays and with a prominent ventral hump; medio-lateral and postero-lateral rays similar and approximate; externo-dorsal ray more slender than but equal in length to the externo-lateral ray, arising at the base of the dorsal ray. Dorsal ray divided near its extremity into two simple branches. Spicules filiform, equal, from 470 to 490 μ long, each with a finely striated wing; spicules united at their tips. Gubernaculum present, 67μ long.

FEMALE up to 30 mm. long, form similar to that of male. Buccal capsule much broader than deep; internal transverse diameter 370 μ; depth of chitinous portion 280 μ; wall of capsule 20 μ thick. Buccal teeth shorter and blunter than in male; largest tooth 60 μ high. Head papillae as in male; slightly more anterior in location. Esophagus 960 μ long, clavate, 130 μ thick at anterior end, 250 μ thick just before the esophago-intestinal valve; nerve ring at middle of length of esophagus; cervical papillae opposite the thickest portion of the esophagus; excretory pore near esophago-intestinal valve. Vulva just before the middle of the body; lips prominent. Anus just before the slender caudal appendage. Eggs 72 μ × 42 μ, thin shelled, with a small operculum at one pole.

Host.—*Buteo borealis.*

Location.—In posterior thoracic air sacs.

Geographic distribution.—Fairfax County, Virginia, U. S. A.

Type and allotype.—U. S. N. M., Helm. Coll. No. 25969; paratypes No. 25970. One pair deposited in the British Museum of Natural History.

Cyathostoma coscorobae n. sp. (pl. 4, fig. 43, 45, 46).

Syngamus trachealis Wied. (pars), Fox, 1923, Disease in captive wild mammals and birds, p. 550.

MALE 5.5 mm. (estimated) long, anterior portion attenuate. Buccal capsule with straight sides, its lumen 83 μ in diameter, 100 μ deep, lateral walls 13 μ thick. Buccal teeth six in number, alternate teeth about twice size of others, large teeth 33 μ high. Head papillae, as far as could be determined, as in *C. americana*. Esophagus about 700 μ long, greatest width just before the esophago-intestinal valve, where it is 150 μ in diameter. Nerve ring just before the middle of the length of esophagus. Cervical papillae and excretory pore not seen. Cuticle with an exceedingly fine transverse striation which is evident only near ruptured portions and which may be the result of the rupture.

Caudal bursa well developed; when spread 720 μ in transverse diameter. Ventral rays similar, slender, and approximate, about 105 μ long; lateral rays parallel and continuous throughout their length; externo-lateral ray the shortest (135 μ long); medio-lateral and postero-lateral rays 150 μ and 210 μ long, respectively; externo-dorsal ray 165 μ long, arising near the base of the main trunk of the dorsal ray, which is 150 μ to its bifurication; branches of dorsal ray sinuous, each with three terminations, as in *C. bronchialis*. Spicules filiform, from 660 μ to 720 μ long, each with a finely striated wing; spicules united at tips. Gubernaculum present, 92 μ long.

FEMALE about 20 mm. long. Buccal capsule trapezoidal in optical section, 210 μ in depth, its walls 30 μ thick, diameter of the lumen at base 225 μ, at apex 285 μ. Teeth six in number, proportionately lower than in male, the highest teeth 60 μ. Esophagus 900 μ long, its greatest diameter 255 μ just before the esophago-intestinal valve; nerve ring at the anterior, two-fifths of the length of the esophagus. Cervical papillae and excretory pore not seen. Vulva at about the middle of the body length; vagina very short; uteri divergent. Tail acute; anus 225 μ before tip. Eggs oval, 80 μ long by 50 μ in transverse diameter, with a minute operculum at the slightly smaller end.

Host.—*Coscoroba coscoroba* (Coscoroba swan).

Location.—Trachea.

Geographic distribution.—South America (from captive bird in Philadelphia Zoölogical Park).

Type and allotype.—U. S. N. M., Helm. Coll. No. 26128; paratypes in collection of the Zoölogical Society of Philadelphia.

The specimens upon which the above description is based were collected by Herbert Fox and his associates from a Coscoroba swan which died July 26, 1919. The specimens appear to have been left for some time in water with the result that all have ruptured. Because of this, it has been impossible to make accurate measurements of the total length or of the position of the vulva.

CYATHOSTOMA SP.

In the collection of worms received from Philadelphia there are three female Cyathostoma sp. from the blue-winged teal (*Querquedula querquedula discors*). One of the three specimens is in condition for study and belongs to a species of the second group, in which the vulva is at or near the middle. There is nothing to separate the species from *C. lari* Blanch. except that the hosts are widely separated phylogenetically.

NOTE

Since the completion of the present paper, L. Gedoelst [3] has described a new Syngamus, *S. hippopotami*, from the hippopotamus, collected at Nyangwa, Belgian Congo. The worms, judging from the photographic illustration are of the *laryngeus* type; that is, the posterior half of the female is markedly narrower than the anterior half. If this supposition is correct, the species need only be compared with *S. laryngeus* Raill. *Syngamus hippopotami* Gdlst. is a much larger species, the females ranging from 24 to 34 mm. The spicules of the male are smaller and are of two sizes, the shorter being from 12–18 μ, the larger from 18–24 μ. The dorsal ray is bifurcate clear to the base instead of being single, and occasionally each branch bears a small lateral subray. In these, as well as in other characters, the species is quite distinct from *S. laryngeus*. In comparison with species other than *S. laryngeus*, *S. hippopotami* is readily distinguished by the extremely small spicules.

[3] GEDOELST, L. UN SYNGAME PARASITE DE L'HIPPOPOTAME. Ann. Par. 2: 307–311, illus. 1924.

COOPERIA BISONIS, A NEW NEMATODE FROM THE BUFFALO[1]

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INTRODUCTION

In material collected during postmortem examinations of buffalo at the National Buffalo Park, Wainwright, Canada, in January and February, 1923, by S. Hadwen and A. E. Cameron, and sent to the Bureau of Animal Industry, U. S. Department of Agriculture, there were numerous specimens of Cooperia which proved to be a new species. It is to this nematode that Cameron[2] refers in his "Notes on Buffalo" and which, through some lapsus, was designated *Haemonchus ostertagi*. The present writer, however, does not find that any of the specimens attain the length described by him—14 mm. for the female. Cameron notes as the pathological picture an inflammation of the mucous membrane of the fourth stomach and duodenum of the buffalo.

FIG. 1.—*Cooperia bisonis*. Anterior extremity

SPECIFIC DIAGNOSIS

Cooperia: Cuticle of head (fig. 1) markedly expanded, followed by a sharp constriction and then a second flaring out for a short distance. Cuticle shows deep transverse striations throughout dilated region. The nerve ring surrounds the esophagus at a point about three-fourths of the length of the esophagus from its anterior end.

MALE.—The male is 7.2 to 7.7 mm. long by 175 to 190 μ in maximum width just anterior to the bursa. Head 37 μ wide, narrowing down to 33 μ posterior to the cuticular dilation. Esophagus 357 to 382 μ long by 20 μ in diameter throughout about three-fourths of its length, increasing to 33 μ in maximum diameter near the posterior end. Free margins of bursa delicately striated. Lateral lobes of bursa (figs. 2 and 3) curved inward; median lobe divided in two by a small median incision. The externodorsal ray is the most slender of the paired rays, the others thicker in the following order: The ventroventral, postero-lateral, medio-lateral, latero-ventral, and lastly the externolateral, which is by far the thickest. The total length of the dorsal ray is 249 to 257 μ; the stem (149 to 158 μ long to the point of division) bifurcates to form a U-shaped arch. Each branch soon after its division from the main stem gives off exteriorly a thick, thumblike projection about 50 μ long, the free distal half being bent ventrally.

FIG. 2.—*Cooperia bisonis*. Bursa of male; lateral view

[1] Received for publication June 30, 1924; issued June, 1925.

[2] CAMERON, A. E. NOTES ON BUFFALO: ANATOMY, PHYSIOLOGICAL CONDITIONS AND PARASITES. Vet. Jour. 79: 331-336. 1923.

There is little or no tapering of the terminal dorsal branches; near their extremities they are cleft to give off, exteriorly, delicate finely pointed processes; they themselves end bluntly. The spicules (fig. 4) are 224 to 240 μ long and 19 to 24 μ wide; they are of simple structure. The muscular attachment at their proximal end shows plainly as a balloonlike expansion. The telamon (figs. 5A and B) is very elaborate. On the dorsal surface of the genital cone it consists of a strongly chitinized U-shaped structure, and posterior to this

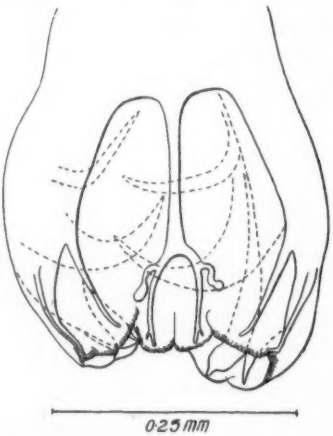

FIG. 3.—*Cooperia bisonis*. Bursa of male; dorsal view

two pairs of papilliform bodies in the lateral fields, and in the median line a process apparently ending in a papilla, extending backward between two chitinized basketlike structures. Masses of granular material extend laterally on both sides of the cone. On the ventral surface there is a prominent chitinized structure consisting of two large, movable, projecting plates. Between them on the posterior surface is a strongly developed rounded body. It is directly below this body, between it and the papilliform process dorsal to it, that the spicules protrude. It is possible that the large, movable projections are clasping organs; the papillae may be sensory in nature; the basketlike structures may serve to support and guide the spicules, and the granular material may act as a cement plug.

FIG. 4.—*Cooperia bisonis*. Spicules

The telamon has not been previously described for Cooperia; it is evidently the most elaborate of any of the telamons studied up to this time. Hall[3] first described this structure in *Hyostrongylus rubidus* and *Ornithostrongylus quadriradiatus*. Lane[4] has noted its character in *Necator congolensis* and Goodey[5] has recently made a detailed study of it in *Oesophagostomum dentatum*. It is evidently present in cylicostomes of the horse, as figures by Kotlán[6] and more recently by Smit and Notosoediro[7] clearly show such supporting structures, although the authors do not call attention to them as telamons. It seems quite probable that this structure will prove to be of value as a definite specific character when it is more widely studied.

FEMALE.—The female is 8 to 9.5 mm. long by 157 to 257 μ in maximum diameter just anterior to the vulva. Head 38 to 41.5 μ wide, narrowing down to 30 to 33 μ posterior to the cuticular dilation. Esophagus 378 to 431 μ in length by 21 μ in smallest diameter, increasing to 33 μ near the posterior end. The vulva (fig. 6) is situated 1.9 to 2.4 mm. from the posterior

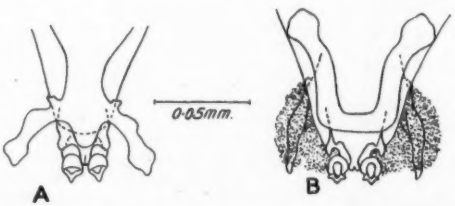

FIG. 5.—*Cooperia bisonis*. Telamon. A, ventral view; B, dorsal view

[3] HALL, M. C. TWO NEW GENERA OF NEMATODES, WITH A NOTE ON A NEGLECTED NEMATODE STRUCTURE. Proc. U. S. Nat. Mus. 59: 541–546, illus. 1921.

[4] LANE, C. SOME STRONGYLATA. PARASITOLOGY 15: 348–364. 1923.

[5] GOODEY, T. THE ANATOMY OF OESOPHAGOSTOMUM DENTATUM (RUD.) A NEMATODE PARASITE OF THE PIG, WITH OBSERVATIONS ON THE STRUCTURE AND BIOLOGY OF THE FREE-LIVING LARVÆ. Jour. Helminthology 2: 1–15. 1924.

[6] KOTLÁN, SÁNDOR. ADATOK A LÓVAKBAN ÉLŐSKÖDŐ STRONGYLIDÁK ISMERETÉHEZ. Néhány új Cylicostomum-faj lovak vastagbeléből. Állat. Lapok 43: 71, 85–86, illus. 1920.

[7] SMIT, H. J., and NOTOSOEDIRO, R. NOG EENIGE STRONGYLIDEN VAN HET PAARD OP JAVA. Nederland. Ind. Bl. Diergeneesk. en Dierenteelt 34: 62–68, 224–232, 446–455, illus.; 1923; 35: 29–36, illus. 1924.

end of the body. It has chitinous lips protruding obliquely to the axes of the body; it is covered by a large projecting linguiform process 166 to 177 μ in length, extending backward. The width of the body shows a marked increase just anterior to the vulva, being 157 to 257 μ at this point; posterior to the vulva is a sharp diminution in diameter, it being only 91 to 116 μ wide. The combined length of the muscular ovejectors, including the sphincters, is 532 to 747 μ. The eggs appear strikingly large in proportion to the width of the worm; they are 91 to 99 μ long by 41 to 49 μ wide and are in the morula stage of development when they reach the vulva. The anus (fig. 7) is situated 174 to 190 μ from the posterior extremity. The body gradually narrows posterior to the anus to form a delicately pointed tail bearing at its tip a small bulbous swelling. Toward the extremity the transverse striations of the cuticle are deeper than elsewhere, giving the appearance of pseudo-annulations.

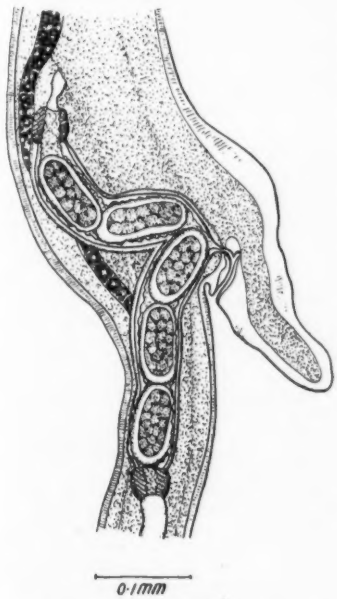

FIG. 6.—*Cooperia bisonis*. Vulva region of female

Host.—Bison bison.

Location.—Fourth stomach and duodenum.

Locality.—Wainwright, Alberta, Canada.

Type material.—Bureau of Animal Industry, Helminthological Collection, No. 25516.

Cooperia bisonis is most closely related to *C. oncophora*, but differs from it in showing a more marked thickening of the body in the region of the vulva; in the vulva and anus being somewhat farther from the posterior end of the body; in the large linguiform process overlapping the vulva; in the larger size of the eggs, and in the ventral branches of the dorsal rays originating near the junction of the latter with the stem, rather than from the middle of them. The size of the eggs of *C. bisonis* (91 to 99 μ) exceeds that given in the generic description (60 to 80 μ), so that the latter will have to be modified to include this species.

FIG. 7.—*Cooperia bisonis*. Posterior extremity of female

The points of distinction of the five species of Cooperia found in ruminants are covered in the following key:

1. Total length of dorsal ray 70 to 75 μ; spicules of male less than 200 μ long; vulva of female situated not more than 1.5 mm. from posterior end of body 2
   Total length of dorsal ray 180 μ or more; spicules more then 200 μ long; vulva situated 1.6 mm. or more from posterior end of body 3
2. Branches of dorsal ray curved in shape of a lyre; combined length of muscular ovejectors 375 to 500 μ; chitinous lips of vulva elongated transversely ..... *C. curticei*
   Branches of dorsal ray nearly straight and parallel; combined length of muscular ovejectors 250 to 275 μ; chitinous lips of vulva elongated longitudinally .. *C. punctata*
3. Vulva covered by a large projecting linguiform process; eggs 91 to 99 μ long by 41 to 49 μ wide; spicules 224 to 240 μ long .. *C. bisonis*
   Vulva not covered by a large projecting linguiform process; eggs 60 to 80 μ long by 30 to 36 μ wide; spicules 240 to 300 μ long 4
4. Total length of dorsal ray 220–400 μ; branches of dorsal ray horse-shoe or U-shaped, with cleft tips; combined length of muscular ovejectors 700 μ ..... *C. oncophora*
   Total length of dorsal ray 180 μ; branches of dorsal ray close together, parallel and uncleft; combined length of muscular ovejectors 300 μ ..... *C. pectinata*

SOME PHYSICAL AND CHEMICAL PROPERTIES OF XANTHOPHYLL AND THE PREPARATION OF THE PURE PIGMENT[1]

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INTRODUCTION

Xanthophyll when found in plant materials is associated with carotin. It is present in all green plants along with carotin, but in larger quantity.

Much has been published regarding the carotinoids, but as methods of isolating the pigments have differed somewhat from time to time, it is difficult to say whether xanthophyll was or was not present in any given plant or animal material reported in the literature. It has been definitely established that xanthophyll or its isomer is present in hen eggs and in the body fat of chickens (*7*),[2] and also in yellow corn. Gill (*3*) claimed that a relatively small amount of the pigment in yellow corn is carotin, although Palmer and Kempster (*7*) and Palmer and Eckles (*6*) showed that yellow corn is rich in xanthophyll and that a little of the pigment is found in hemp seed, barley, gluten feed, and red corn.

Some of the work reported in the literature has not been done with sufficient accuracy to warrant a statement regarding the amount of xanthophyll present in certain plant and animal substances. Very often, too, xanthophyll is confused with carotin and vice versa, and sometimes it is known under another name, the name given being entirely misleading, for it is only recently that reliable methods of procedure have been worked out for the separation (*12*) and complete identification of these two pigments.

Even in the more recent literature statements regarding the carotinoids are often difficult to explain. Gill (*3*) states that none of the pigments of yellow corn, mustard, and orange peel are extracted from a petroleum ether solution by 80 to 90 per cent alcohol, although large amounts are absorbed by calcium carbonate. Since none of the pigments are extracted by the alcohol it would appear that xanthophyll is not present, and again, since adsorption by calcium carbonate is large, it appears that the pigment is xanthophyll. Another instance of seemingly contradictory results can be found in the work of Palmer and Eckles (*6, p. 362*), if the standards set by Tswett (*10*) regarding xanthophyll are accepted; the xanthophyll-like constituents of yellow corn were not adsorbed by calcium carbonate from a carbon disulphide or a petroleum ether solution and, on the other hand, the pigments could be completely extracted from a petroleum ether solution by 80 per cent methyl alcohol.

Here are two cases of xanthophyll behavior which do not agree with the generally accepted idea of its behavior. The xanthophyll reported by Gill could not have been xanthophyll, since it was not extractable by alcohol, while that reported by Palmer and Eckles would be xanthophyll, according to the standard set by Willstätter, since it was extractable by 80 per cent alcohol. These two cases of conflicting statements make it quite plain that there is need of work on the Tswett (*10*) adsorption method in connection with the distribution of carotin and xanthophyll between petroleum ether and alcohol. It is very desirable that the different xanthophylls, as described by Tswett, be obtained in crystalline form so that the pure pigments, if they really exist, may be identified.

Palmer and Eckles also have shown how the xanthophyll-like fraction from carrots produces several adsorption bands when the carbon disulphide solution is passed through a calcium carbonate adsorption column. The pigment of Zone I appears entirely unlike the others, for it is completely adsorbed by calcium carbonate from a carbon

[1] Received for publication June 13, 1924; issued June, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 584.

disulphide solution, while the others are not adsorbed but pass through if more carbon disulphide is added. It appears as if several xanthophylls were present.

ISOLATION AND PURIFICATION OF XANTHOPHYLL

In order that the purity of the preparation of xanthophyll used in the work herein described may be unquestioned, and also to aid those who are desirous of obtaining the pure pigment, it is considered advisable to describe very fully the method which has been adapted from those described by Willstätter and Stoll (*12*).

The best source of xanthophyll is dried green leaves. The leaves may be kept for several months and the xanthophyll extracted when desired. Although the leaves of almost any green plant would be suitable, cowpea leaves were used in this investigation. The leaves are carefully spread out to dry, away from sunlight. A large oven with screen trays may be used, or a large screen on which the leaves may be stirred. Room temperature is hardly sufficient, but for drying small quantities it serves very well. Rapid drying is preferable, though great care must be used not to get the temperature too high (above 40° C.), since too high a temperature destroys a large percentage of the pigments present in the leaves. The drying will take from 12 to 24 hours, or a little longer if large quantities of leaves are used.

As soon as the leaves are dried they are placed in a large pebble mill—the mill in use in the Office of Soil Fertility Investigations is about 38 cm. in diameter and 45 cm. in length—and ground 12 to 24 hours. When ground to powder, they are removed from the mill and placed in either glass or tin containers. Since the carotinoid pigments oxidize very readily, the material should be kept in sealed containers. Leaf material may be kept in this manner for several years, and used as desired for the preparation of xanthophyll.

The method here described will be suitable for obtaining 0.5 to 0.8 gm. of pure xanthophyll from 2 kgm. of the dried leaves. A quantity of leaves sufficient to produce this amount of xanthophyll is about all that can be easily handled with ordinary laboratory apparatus, and even then some special apparatus will be desirable.

Pure chlorophyll and carotin may also be prepared (these processes will not be described here) at the same time from the extract by a little more labor, if the worker desires the pigments. The method of preparing pure xanthophyll is essentially that given by Willstätter and Stoll, but certain modifications which are very necessary for good results will be added.

Two kilograms of the leaf powder are placed upon a large porcelain Büchner funnel (25 to 30 cm. in diameter) to which a filter paper has first been fitted. The leaf powder is spread out carefully but not compressed. The funnel has previously been fitted to a 4-liter filter flask which is connected to the vacuum pump. At first 80 per cent acetone is allowed to soak into the dry leaf powder, which is stirred with a spatula or glass rod in order to hasten this part of the process. As soon as the 80 per cent acetone has thoroughly moistened the leaf powder, suction is applied and more acetone is poured on. If the leaves are compressed or suction is applied before the leaf powder is thoroughly moistened with the aqueous acetone, filtration will be greatly retarded. The green acetone extract is sucked off and more acetone added in liter portions until approximately all of the leaf pigments have been extracted. About 6 to 8 liters of 80 per cent acetone will be required for the extraction, which should be completed in from 30 to 60 minutes.

About half of the acetone extract is now poured into a 7-liter separatory funnel and 2 liters of distilled petroleum ether are added. The whole is shaken thoroughly, and about 250 c. c. of distilled water added in order to cause a separation of an aqueous acetone layer, which is drawn off. The remainder of the acetone extract is added to the separatory funnel, shaken, and the acetone layer is again separated by adding another 250 c. c. portion of water. The aqueous acetone solution contains the yellow flavones which are soluble cell sap pigments.

To the petroleum ether solution remaining in the funnel 1,000 c. c. of 80 per cent (by volume) acetone is added and shaken thoroughly. This solution is allowed to stand a minute and run off. The impurities are thus removed and only a very little of the xanthophyll is lost. The acetone is now partially removed by four successive washings, each with 250 c. c. of distilled water. Four washings are sufficient, for if more of the acetone is washed out the xanthophyll will crystallize during subsequent washings with methyl alcohol and the separation of the xanthophyll from the chlorophyll will then become much more difficult.

To separate the xanthophyll from the petroleum-ether acetone solution 1 liter of 80 per cent methyl alcohol is added, the solution is shaken three times, and the yellowish-green methyl alcohol layer is drawn off each time. If the last extract is still quite yellow more extractions will be necessary. The methyl alcohol extracts are combined and placed in a 7-liter separatory funnel or a 5-gallon water bottle. The petroleum ether solution by further treatment may be used for the preparation of chlorophyll and carotin.

The xanthophyll is extracted from the methyl alcohol by mixing the combined xanthophyll extracts with 4 to 6 liters of ether. This solution is shaken thoroughly, and a saturated aqueous sodium chloride solution equal in amount to the methyl alcohol is added. The ether layer containing the xanthophyll and traces of chlorophyll will separate above and the aqueous methyl alcohol layer below. The aqueous methyl alcohol layer is then run off. About 2 liters of ether are added to this aqueous methyl alcohol solution and shaken again; more of the salt solution is added and allowed to stand a half hour, when the ether layer will have separated. The ether extraction is repeated if necessary. If a 5-gallon bottle is used the aqueous methyl alcohol layer can be siphoned off into another bottle and then the ether solution of xanthophyll may be poured into a separatory funnel, where the last traces of methyl alcohol and acetone may be washed from the ether.

The ether solutions of xanthophyll are all combined in a 4 or 6 liter separatory funnel and washed several times with water to remove alcohol and traces of acetone. The yellowish-green ether solution which contains the xanthophyll and some chlorophyll *b* is now dried by shaking with anhydrous sodium sulphate (100 to 200 gm.). After pouring the ether solution from the sodium sulphate it is shaken with 50 c. c. of concentrated methyl alcoholic potash.

Saponification is best carried out at room temperature in a gallon bottle or in a separatory funnel and is done in order to remove chlorophyll *b*, the potash salt of which is soluble in water. The water soluble salt of chlorophyll *b* is now washed from the ether, 100 to 200 c. c. of water being used for each of several washings. The xanthophyll remains in the ether.

The ether solution is dried with anhydrous sodium sulphate, then filtered and evaporated (using diminished pressure toward the last) to about 25 c. c. on a water bath at a temperature not exceeding 50° C. The xanthophyll should not remain in contact with ether long, for experiments reported below show that ether will oxidize the xanthophyll and cause a lower yield. The concentrated ether solution of xanthophyll is mixed with 200 to 300 c. c. of absolute methyl alcohol while the solution is still in the water bath and the ether is removed under reduced pressure. The methyl alcohol solution is filtered through a Büchner funnel with suction while hot to remove impurities and the last traces of ether. The concentrated solution of xanthophyll in methyl alcohol is now allowed to stand in the ice chest for a day or more, when beautiful crystals of xanthophyll will have separated. In order to make the separation complete a little water is added, when the xanthophyll will form crystalline aggregates which are arranged radially. On standing for a day or two, the aggregates gradually change to the usual form of leaf clusters. The yield of xanthophyll when separated from the above mother liquor by filtering and washing with petroleum ether to remove traces of fats, etc., is 0.5 to 0.8 gm., or more. The xanthophyll must be further purified, however, if it is to be used for accurate work.

Xanthophyll takes up ethyl or methyl alcohol in crystallizing and may be freed of this by precipitating from chloroform with petroleum ether. Its further purification is completed as follows: The crystals of xanthophyll, obtained as described above, are carefully dissolved in boiling absolute methyl alcohol. This is done by placing the crystals in the alcohol and bringing to a boil on the water bath for a short time (1 to 5 minutes). This solution is filtered by suction to remove any undissolved crystals. The saturated solution is then placed in the ice box over night or longer. The crystals will separate in much larger plates than before and have an unusually strong surface luster. They are filtered off by suction, washed with low-boiling petroleum ether, recrystallized a third time, and often a fourth or fifth time, from methyl alcohol and then dissolved in chloroform. The chloroform solution is concentrated by suction and warming until xanthophyll begins to separate; the solution is then warmed to bring all of the material into solution and immediately 200 to 400 c. c. of redistilled low-boiling petroleum ether is added slowly. A yellowish

precipitate (the depth of color depends upon several factors) forms almost immediately and can be collected on a hardened filter. The precipitate is washed quickly with pure low-boiling petroleum ether and dried in a vacuum desiccator at once. The drying is only for the purpose of removing the petroleum ether. The melting point should now be 173 to 174° C.

Drying is accomplished in 15 to 20 minutes, and weighings of xanthophyll are made at once for all of the solutions needed. Any xanthophyll remaining may be stored in the ice box in a flask containing petroleum ether, methyl or ethyl alcohol. If more xanthophyll is required later for any purpose the xanthophyll is recrystallized from methyl alcohol and is reprecipitated from chloroform and petroleum ether. In this way a pure product is assured each time. Pure anhydrous ether is the best solvent for xanthophyll, but as deterioration is greatest in this solvent readings must be made at once. If xanthophyll is dissolved in absolute alcohol, difficulty is often experienced in getting the last traces of the pigment into solution. It would seem that pure xanthophyll might be obtained by precipitating several times from chloroform with petroleum ether, as is the case when carotin is purified by dissolving in carbon disulphide and then recrystallizing from petroleum ether. Observations have shown, however, that xanthophyll can not be purified in this manner. To get the purest xanthophyll preparations, spectrophotometer readings show that crystallizations must first be made from methyl alcohol and that the xanthophyll must then be precipitated from chloroform by the addition of petroleum ether. Melting-point determinations are not as good a criterion of purity as are spectrophotometric observations, as data given in a former paper (*8*) show.

CHEMICAL AND PHYSICAL PROPERTIES OF XANTHOPHYLL

SOLUBILITY

Solubility determinations were made, using petroleum ether which fractionates at 50 to 55° C., absolute ethyl alcohol (99.7 to 100 per cent), absolute methyl alcohol, and anhydrous ether which had been specially prepared by washing U. S. P. ether with water four times, distilling over calcium chloride and then allowing to stand over sodium for a week, finally distilling immediately before use.

The xanthophyll used here was prepared fresh in every case as described in the preceding section. Solubility tests were made in a water bath kept at 25° C. and the pigment content was determined by means of the spectrophotometer and the mercury line 435.8 mμ. The stability of xanthophyll in absolute ethyl alcohol, in absolute ether, in U. S. P. ether, and in petroleum ether was also tested in this manner, since the results obtained in quantitative work depend upon the stability of xanthophyll in solution.

The solubility of xanthophyll differs considerably from that of carotin, and this property is utilized in the separation of the two pigments. The solubilities were determined in practically the same manner as were the solubilities of carotin (*9*).

Table I shows the solubility of xanthophyll in petroleum ether. The transmittancies given for all of the solvents are evaluated from graph 11, Figure 2 of a previous paper (*8*), for the quantitative determination of xanthophyll in solution. The determinations when averaged show the solubility to be 9.51 mgm. per liter.

TABLE I.—*Solubility of xanthophyll in petroleum ether (B. P. 50 to 55° C.) at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	5	22	0.174	9.10
	5	70	.140	10.15
2	5	42	.174	9.10
	5	56	.184	8.80
3	5	60	.133	10.40

The results for absolute alcohol do not check as well as do those for the other solvents. It would seem that the later experiments reported in Table II were the best, for impurities in xanthophyll tend to increase the amount of material held in solution. The average of the last two experiments has been taken as the solubility (201.5 mgm. per liter) of xanthophyll in absolute ethyl alcohol.

The solubility determinations in absolute methyl alcohol are given in Table III. Only the data for experiments Nos. 2, 3, and 4 have been averaged for ascertaining the solubility in methyl alcohol, as there is some indication that the first experiment gave too high a result. This average shows the solubility of xanthophyll in absolute methyl alcohol to be 134.9 mgm. per liter.

TABLE II.—*Solubility of xanthophyll in absolute ethyl alcohol at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	100	16	0.0696	276
	100	47	.0742	271
	100	64	.0822	258
2	100	22	.0846	256
	100	46	.0952	243
	100	94	.1020	237
3	50	42	.0192	206
	50	56	.0214	199.5
4	50	60	.0215	199

TABLE III.—*Solubility of xanthophyll in methyl alcohol at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	200	22	0.4420	166
	100	70	.2620	138
2	50	42	.0754	134
	50	56	.0740	135
3	50	60	.0816	129
4	50	60	.0649	141.5

Willstätter and Mieg (*11*) state that the oxidation product of xanthophyll is very easily soluble in methyl alcohol. Tests made during the course of this investigation confirm this statement. They further state that the addition of ether to a solution of oxidized xanthophyll in methyl alcohol will cause the oxidized xanthophyll to separate as a white powder. These statements assist materially in understanding the solubility of xanthophyll in the four solvents used as shown in the tables. The tables show that the alcohols would tend to exhibit too great a solubility when impure xanthophyll is used if the impurity is oxidized xanthophyll; and, on the other hand, the solubility in ether would not be greatly affected, for oxidized xanthophyll is very difficultly soluble in ether. Petroleum ether apparently behaves as does ether, for Table I does not show any great difference in solubility in the sample used in experiment 1 and experiment 2.

The average of the determinations (Table IV) shows the solubility of xanthophyll in ether to be 952 mgm. per liter.

In Table V the solubility of xanthophyll and carotin in these solvents is compared.

Willstätter and Stoll (*12*) state that 1 gm. of xanthophyll dissolves in 700 c. c. of boiling, or in 5 liters of cold,

TABLE IV.—*Solubility of xanthophyll in ether at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	500	22	0.151	975
	500	46	.161	945
	500	94	.155	965
2	500	42	.174	910
	500	56	.156	965

TABLE V.—*Solubility of xanthophyll and carotin*

Solvent	Milligrams per liter	
	Xanthophyll	Carotin
Petroleum ether [a]	9.5	626.0
Absolute alcohol (ethyl)	201.5	15.5
Absolute alcohol (methyl)	134.9	[b]
Pure ether (anhydrous)	952.0	1,005.0

[a] The boiling point of the petroleum ether used was 50 to 55° C. for xanthophyll and 35 to 50° for carotin.

[b] Nearly insoluble.

methyl alcohol; in ethyl alcohol it dissolves more easily; 1 gm. dissolves in 300 c. c. of boiling ether and it is so insoluble in petroleum ether that the solvent is not even colored.

From Table V it is seen that carotin and xanthophyll have nearly the same solubility in pure ether. At 25° C. xanthophyll is 13 times as soluble as carotin in absolute ethyl alcohol. The greatest difference in solubility of the pigments in the same solvent is observed in petroleum ether, which dissolves 60.5 times as much carotin as it does xanthophyll.

STABILITY

Four solvents were chosen in which to test the keeping qualities of xanthophyll. All of these are or may be used in the isolation of the pigment from plant materials. The solvents used were: Absolute ethyl alcohol, pure ether, U. S. P. ether as it comes in the container, and redistilled petroleum ether (B. P. 50° to 55° C.).

A solution of xanthophyll (0.210 gm. per liter) was made in freshly distilled anhydrous ether, and of this 10 c. c. portions (2.1 mgm.) were carefully evaporated to dryness in vacuum at room temperature, the solvents were added immediately and

the volumes of the absolute ethyl alcohol, ether, and U. S. P. ether solutions were made up to 250 c. c. In the case of the petroleum ether solution it was necessary to bring the pigment into solution with 20 c. c. of absolute alcohol and then the volume was made up to 200 c. c. by the addition of petroleum ether. These solutions of xanthophyll were then stored at a temperature of 10° to 20° C. and determinations, both colorimetric and spectrophotometric, were made on the solutions. The data are given in Table VI.

The solutions were made up on March 31 and were kept in the ice box, except for short periods when the determinations were being made. Table VI and Figure 1 show clearly that xanthophyll oxidizes quite rapidly in ether solutions, the decomposition being about the same whether the ether was freshly distilled or not.

The oxidation of the carotinoids in ether agrees well with the development of ethyl peroxide $(C_2H_5)_2O_3$ in ether as described by Clover (*1*). There probably is a correlation between the development of peroxide and the oxidation of the carotinoids.

Petroleum ether and absolute alcohol solutions kept well, showing practically no oxidation during the time that they were in storage.

The keeping quality of carotin in the various solvents was quite similar to that of xanthophyll. In other words, carotin and xanthophyll rapidly oxidize when kept in ether solutions, while both carotinoids are stable when kept in a cool, dark place either in petroleum ether or in absolute alcohol, absolute alcohol apparently being the medium in which both pigments are most stable.

OXIDATION OF XANTHOPHYLL AND CAROTIN

The rate of oxidation of xanthophyll has been variously reported in the literature. This is also true of carotin. The oxidation of the pure pigments will be discussed first and then the oxidation of the impure pigments, that is, those that are still present in the leaf tissue.

The melting point of the pigments is lowered when the carotinoids oxidize. On account of this fact it was attempted to ascertain the comparative rate of oxidation of the two yellow pigments by determining their melting points at various intervals of time when the powders were exposed to the air at room temperature. The results were entirely unsatisfactory, for pure xan-

TABLE VI.—*Keeping qualities of xanthophyll in solution*

Solvent	Date	Colorimetric data				Spectrophotometric data	
		Colorimeter readings, depth in mm. using Lovibond slides			Colorimeter determinations, solution undiluted, mgm. per liter, average results	Transmittancy after solutions were diluted 5 times	Spectrophotometer determinations, mgm. per liter
		5	10	20			
Absolute alcohol	Apr. 8	3.4	5.3	9.2	7.53	0.202	8.30
Ether	do	3.3	5.6	9.3	7.44	.212	8.00
U. S. P. ether	do	3.0	5.2	9.0	7.96	.190	8.60
Petroleum ether	do	2.6	4.1	7.1	9.66	.148	9.90
Absolute alcohol	Apr. 24	3.2	5.5	8.9	7.73	.198	8.28
Ether	do	4.3	7.1	12.5	5.83	.277	6.65
U. S. P. ether	do	3.8	6.2	10.7	6.63	.222	7.75
Petroleum ether	do	2.7	4.2	7.2	9.40	.146	10.00
Absolute alcohol	May 24	3.1	5.0	7.7	8.40	.210	8.12
Ether	do	7.8	12.3	17.1	3.63	.457	4.08
U. S. P. ether	do	5.7	9.1	14.5	4.53	.359	5.30
Petroleum ether	do	3.0	4.3	7.6	8.90	.148	9.85
Absolute alcohol	July 21	3.8	5.4	8.7	7.40	.207	8.16
Ether	do				(a)	[b] .286	1.30
U. S. P. ether	do				(a)	[b] .292	1.26
Petroleum ether	do	3.2	5.1	9.0	7.86	.173	9.10
Absolute alcohol	Sept. 11	3.4	5.3	9.2	7.53	.214	7.96
Petroleum ether	do	3.5	5.7	9.6	7.23	.205	8.20

[a] Too dilute to estimate.

[b] Undiluted.

thophyll is obtained in a very finely precipitated form, whereas carotin is in the form of crystals. To begin with, then, the substances are not in the same state of subdivision. Furthermore, when carotin crystals are allowed to oxidize the outer portion of the crystal oxidizes first and, consequently, melts first in a melting point tube. There is a melting point (which apparently varies with the degree of oxidation) for the oxidized portion of the crystal, and there is also a melting point for the unoxidized portion. This was ascertained by viewing the melting point tubes, after heating so as to melt the outer surface of the crystals, under the microscope. It is obvious that such determinations made on the two pure pigments would be valueless in regard to comparative rates of oxidation. Pure xanthophyll is apparently more readily oxidizable than pure carotin, for all color disappears in the xanthophyll sample before it does in the carotin sample, although this may perhaps be due to the fact that the substances are in different states of subdivision.

Two possible means of determining the relative rates of oxidation of the two pigments will now be considered. The first deals with the pigments in a dry state and the second with the pigments in solution.

Of a solution (0.210 gm. per liter) of xanthophyll in pure redistilled ether, 5 c. c. portions (each representing 1.05 mgm. of xanthophyll) were placed in small crystallizing dishes. Half of these were kept in a dark box and the other half were placed upon the same box, where they were exposed only to subdued light. All were kept at room temperature and protected from dust. Each workday morning as long as the experiment was run 2 c. c. of ether for each sample was used to dissolve the pigments so as to reduce the effect of the protective action of the oxidized portion and also to dissolve the crystals which might form. The experiment was begun on March 31, but no quantitative readings were made until April 8. The results of these tests on the rate of oxidation of the pigments are given in Table VII. Samples exposed to light showed a much greater rate of oxidation than those kept in the box at room temperature, protected from light. After 36 days about 25 per cent of the original amount of xanthophyll still remained in the sample in the dark box, while only about 1 per cent remained in the sample exposed to the light, as indicated by the spectrophotometric estimations.

Experiments were conducted with carotin similar to those with xanthophyll. The results are reported in

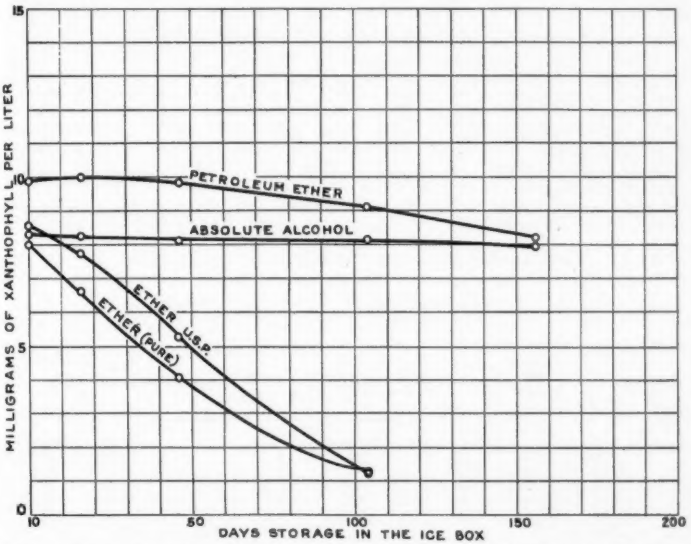

FIG. 1.—Spectrophotometric comparison of the rate of oxidation of xanthophyll in petroleum ether, alcohol, and ether when stored in the ice box at 10° to 20° C.

TABLE VII.—*Oxidation of xanthophyll (in dry form)*

SAMPLE EXPOSED TO LIGHT (ROOM TEMPERATURE)

Days	Date	Spectrophotometric data				Colorimetric data				
		Transmittancy	Volume	Xanthophyll per sample	Xanthophyll unoxidized	Lovibond slide, yellow 5	10	20	Volume	Xanthophyll per sample
			C. c.	*Mgm.*	*Per cent*				*C. c.*	*Mgm.*
0	Mar. 31			1.05	100					1.05
7	Apr. 8	0.324	500	.58	55.2	4.4	7.5	12.3	100	.56
14	Apr. 15	.178	100	.18	17.1	12.2	20.5			(a)
21	Apr. 22	.271	100	.13	12.3	5.0	7.2	13.0	25	.14
28	Apr. 29	.237	25	.037	3.5					(a)
35	May 6	.633	25	.012	1.1					(a)

SAMPLE KEPT IN DARK (ROOM TEMPERATURE)

0	Mar. 31			1.05	100					1.05
7	Apr. 8	0.216	500	.79	75.2	3.1	5.5	9.1	100	.77
14	Apr. 15	.088	200	.50	47.9	4.5	7.1	11.8	100	.58
21	Apr. 22	.012	200	.44	41.9	3.1	4.9	8.0	50	.42
28	Apr. 29	.027	100	.37	35.2	3.1	5.4	8.5	50	.39
35	May 6	.137	125	.25	23.8	2.4	3.8	6.3	25	.26

[a] Too dilute to estimate.

TABLE VIII.—*Oxidation of carotin (in dry form)*

SAMPLE EXPOSED TO LIGHT (ROOM TEMPERATURE)

Days	Date	Spectrophotometric data				Colorimetric data					
		Transmittancy	Volume	Carotin per sample	Carotin unoxidized	Lovibond slide, yellow 5	10	20	Volume	Carotin per sample	Per cent
			C. c.	*Mgm.*	*Per cent*				*C. c.*	*Mgm.*	
0	May 1	0.443	500	0.44	100	1.3	2.1	3.8	25	0.43	100
7	May 8	.129	125	.28	63.6	2.3	3.5	5.9	25	.27	62.8
14	May 15	.238	125	.19	43.1	3.2	5.2	7.8	25	.19	44.1
21	May 22	.401	125	.12	27.3	5.3	8.3	12.6	25	.11	25.5
31	June 1		125			6.0	9.6	14.7	25	.09	20.9
40	June 10	Completely oxidized.									

SAMPLE KEPT IN DARK (ROOM TEMPERATURE)

0	May 1	0.443	500	0.44	100	1.3	2.1	3.8	25	0.43	100
7	May 8	.0813	125	.34	78.4	2.0	3.1	4.5	25	.30	69.7
14	May 15	.0982	125	.32	72.7	2.1	3.1	5.2	25	.28	65.1
21	May 22	.145	125	.26	59.0	2.2	3.7	6.4	25	.25	58.1
31	June 1		125			2.3	3.6	6.2	25	.25	58.1
91	Aug. 1	Completely oxidized.									

Table III. The samples (0.44 mgm. of carotin in each evaporating dish) were treated exactly like those for xanthophyll as described above. Both the colorimeter and the spectrophotometer were used in making the determinations. The xanthophyll was dissolved in 95 per cent alcohol and the carotin in high-boiling petroleum ether; on these solutions the colorimetric and spectrophotometric determinations were made. Carotin is seen to be less readily oxidizable than xanthophyll in light as well as in darkness at room temperature.

Carotin has been shown (*9*) to oxidize quite readily in absolute anhydrous ether. Since the ether was not redistilled to remove certain objectionable impurities this experiment has

been repeated; the results are given in Table IX. This experiment was made in order to test the effect of light upon the oxidation of the yellow pigments in ether as well as to test the keeping qualities of the carotinoids in this solvent. The ether in this case was carefully redistilled to remove peroxides; experiments were run using both carotin and xanthophyll. The rate of oxidation of the two pigments in pure ether under exactly the same conditions ought to serve as a good index of their respective rates of oxidation.

The solutions were made up using pure pigments. One sample of each solution was stored in the ice box and one of each was allowed to stand in the sunlight during part of the day. The amount of each pigment in the two solutions was determined colorimetrically and spectrophotometrically on the dates indicated in Table IX.

Carotin is seen to be more stable than xanthophyll in the dark in the ice box, although the reverse is true in sunlight at room temperature. In each case the ether used was exactly the same and, consequently, the results should be absolutely comparable.

in the case of xanthophyll." The purity of all the pigments used by Ewart has been seriously questioned (*5*). Consequently, his work is of doubtful value regarding the yellow pigments reported here.

In researches on the carotin and xanthophyll content of green and yellow leaves, Goerrig (*4*) found that the yellow pigment content of dried leaves (*Fagus silvatica*) was affected considerably by temperature and light during the drying process, as the following data show:

	30° dark	90° dark	Sunlight, room temperature
Carotin	33	25	17
Xanthophyll	59×2	28×2	55×2

At the higher temperature xanthophyll appears to be more sensitive than carotin, while in sunlight carotin is affected more than xanthophyll.

Willstätter and Stoll (*12, p. 105*) found on the examination of a carotin

TABLE IX.—*Oxidation of pure ether solutions of xanthophyll and of carotin*

Date	In the dark in the ice box			In sunlight at room temperature			
	Dilution	Transmittancy	Mgm. per liter	Per cent unoxidized	Dilution	Transmittancy	Mgm. per liter	Per cent unoxidized
XANTHOPHYLL								
	Times				*Times*			
May 1	10	0.206	16.5	100	10	0.206	16.5	100
May 11	10	.239	14.9	90.3	2	.065	5.7	34.
May 24	10	.343	10.8	65.4	0	.629	.47	2.
July 21	0	.222	1.5	9.4	Oxidized completely.			
CAROTIN								
May 1	5	0.405	4.80	100	5	0.405	4.8	100
May 11	2	.132	4.42	92	0	.364	1.1	22.4
May 24	2	.213	3.34	69.6	Completely oxidized on May 17.			
July 21	0	.375	1.03	21.4				

The oxidation or rate of bleaching of these two carotinoids has been observed by several authors. Ewart (*2, p. 187*) states that "pure samples of xanthophyll oxidize and bleach much more slowly in the light than do impure samples, xanthophyll being less readily oxidizable than chlorophyll and very much less so than carotin," and (*p. 188*) "using watery emulsions of carotin exposed to light and air, the oxidation was much more rapid than

solution (0.0134 gm. in 0.5 liter of petroleum ether containing a little alcohol) stored in a well-stoppered bottle in the dark for three weeks that it had lost no color. A xanthophyll solution (0.0142 gm. in 0.5 liter of ether) quickly bleached; they believed this to be due to impurities in the ether. After two days an ether solution lost 5 per cent and after three weeks 60 per cent of its color. They state (p. 243) that an ether solution of

xanthophyll bleaches with access of air and also very quickly in the dark, much more quickly than carotin. It is not stated whether the carotin was dissolved in ether or alcohol, hence the results may not be comparable.

Observations made have shown that the ether solutions have bleached, while petroleum ether and especially alcohol solutions of pure xanthophyll and pure carotin are apparently stable, as has been shown in this paper. After several weeks the petroleum ether solution of xanthophyll is only slightly bleached.

RELATIVE OXIDATION OF PURE CAROTIN AND PURE XANTHOPHYLL

Ewart states that pure samples of carotin oxidize more easily than do those of pure xanthophyll. Aqueous emulsions of carotin oxidize more rapidly than do those of xanthophyll. Goerrig finds that at high temperature xanthophyll in drying leaves is more sensitive to oxidation than carotin. At room temperature the reverse is true in sunlight. The writer has shown that an ether solution of carotin is more stable at temperatures of from about 10 to 20° C. than is a solution of xanthophyll. An ether solution of carotin is less stable in the sunlight than is a solution of xanthophyll. Carotin in the dry form at room temperature in the light (not direct sunlight) and in the dark is less readily oxidized than is xanthophyll.

In general, it is evident that carotin, in solution or as the pure substance, is more stable than xanthophyll in the dark and more unstable in the light.

Goerrig found that carotin was affected more than xanthophyll when drying leaves were exposed to sunlight. This investigation shows that similar conditions exist in the case of pure carotin and pure xanthophyll pigments when they are in ether solution and exposed to sunlight. Ewart has stated that carotin emulsions bleach more readily than those of xanthophyll in sunlight.

Attention should be called to the fact that the colorimetric and the spectrophotometric readings given above for carotin (Table VIII) do not confirm the following statement made by Palmer and Eckles (*6*): "Spectroscopic bands apparently disappear long before the pigment shows signs of bleaching." In this case the two methods of observation in the work reported here should give far different values (Table VIII) for the amount of carotin present, and the spectrophotometer would give a lower figure. The results in this paper check as closely as could be expected, and so the conclusion is drawn from the work presented that both the spectroscopic and the colorimetric properties of a carotin or a xanthophyll solution vary directly with the pigment content of that solution, which means that the spectroscopic bands disappear only as the solutions show colorimetric signs of bleaching.

SUMMARY

Apparent inconsistencies regarding the behavior of xanthophyll are pointed out in the literature on the yellow pigments.

The isolation and purification of xanthophyll is fully outlined and details of preparation are given.

At 25° C. 9.5 mgm. of xanthophyll dissolve in 1 liter of petroleum ether (B. P. 50 to 55° C.), 201.5 mgm. dissolve in 1 liter of absolute ethyl alcohol, 134.9 mgm. in 1 liter of absolute methyl alcohol, and 952 mgm. in 1 liter of pure anhydrous ether.

Xanthophyll is unstable in ether solutions, is very stable in absolute ethyl alcohol, and is slightly unstable in petroleum ether when kept in the ice box. Carotin is unstable in ether solutions but apparently is perfectly stable in alcohol and petroleum ether.

In the dry state xanthophyll oxidizes more readily than carotin. In solution xanthophyll oxidizes more readily than carotin when kept in the ice box, but when the solutions are kept in the sunlight at room temperature carotin oxidizes more rapidly than xanthophyll. These results are in harmony with those of Goerrig on the oxidation of pigments in drying leaves.

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PHYSIOLOGICAL STUDIES ON CEREALS. III. THE OCCURRENCE OF POLYPEPTIDES AND AMINO ACIDS IN THE UNGERMINATED MAIZE KERNEL[1]

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INTRODUCTION

Up to comparatively recent years the generally accepted idea was that the ungerminated kernel of cereals contains the nitrogen practically only in the form of proteins and nucleoproteins. Having shown in previous papers that the ungerminated kernel of wheat (*Triticum vulgare*) (*8*)[3] and of oats (*Avena sativa*) (*9*) contains in addition to proteins also polypeptides and free amino acids, it was of considerable interest to find out whether or not this holds good for the maize kernel also. This work was prompted not only by the fact that corn (*Zea mays*) is the most important crop in the United States both in acreage and in value (*11*), but also by the consideration that few polypeptides have ever been shown to occur in plant and animal materials and by the further consideration that the nutritive and physiological significance of foodstuffs and feedstuffs will not be fully understood until all their constituents have been determined. It is, therefore, believed that this paper fills a gap in our knowledge concerning the nitrogen compounds met with in the maize kernel.

So far as proteins are concerned, Chittenden and Osborne (*2*) found that the corn kernel contains albumins, globulins, an alcohol-soluble protein (zein), and proteose. The latter, which is found in the extracts of corn meal, may, however, be an artificial product formed by hydrolysis of the above-mentioned proteins. In addition to these proteins, the presence in the maize kernel of glutelin (a protein soluble in dilute alkalis or acids) was later reported by Osborne (*12*). According to Osborne and Clapp (*13*), the proportions in which the proteins occurred in yellow corn meal were as follows: Globulins, albumins, and proteoses, 0.45 per cent; zein (soluble in alcohol), 5 per cent; and glutelin (soluble in alkaline or acid solutions), 3.15 per cent. Similar data have been reported by Johns, Finks, and Paul (*10*). Knowledge is very meager concerning the nonproteins, to say nothing of the complete absence of data with regard to their presence in the different varieties of corn. Owing to the work of Schulze and Castoro (*15*) it is known that the seed of maize contains 0.90 per cent of nonproteins calculated on the basis of the oven-dried seed, or 4.9 per cent if calculated on the total nitrogen content. It is also known from the work of Schulze (*14*) that maize seed contain 0.25 per cent of lecithin. According to Czapek (*3, p. 157*), the proportion of lecithin in the yellow maize seed is 0.25 per cent, while in the white seed it is 0.28 per cent, calculated on the oven-dried seed. In this paper it is shown that the ungerminated corn kernel contains amino acids and polypeptides. While it is true that the proportions in which they occur in the corn kernel are small, they seem nevertheless to be of considerable significance because amino acids are the bricks out of which the great protein structures are built, and for the further reason that the amino acids, being the most reactive material of the cells, are capable of performing important vital functions in the plant and animal organism (*1, p. 61–62*). The polypeptides, too, standing closely to the proteins, no doubt offer the immediate material for the synthesis of proteins. While the maize seed contains great quantities of proteins, the latter are not diffusible. On the other hand, the amino acids, being soluble and diffusible, represent the best material for translocation of the nitrogen to the growing parts of the young seedling, before the proteolytic enzymes in the seed have come into play.

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[2] The writer's thanks are due to J. G. Wangler, junior chemist, for assistance in carrying out the routine work.

[3] Reference is made by number (italic) to "Literature cited," p. 592.

EXPERIMENTAL DATA

MATERIAL USED

Three corn varieties of commercial and agricultural importance were used in the experiments reported in this paper.

The Four County corn variety, which was started with different strains of Silver King, has been bred since 1910 or 1912 by the Four County Grain Improvement Association at Ackley, Iowa, under the direction of the Iowa Agricultural Experiment Station. About 100,000 acres are grown each year in north-central Iowa.

United States selection No. 77, which is a White Dent variety, selected and bred by the United States Department of Agriculture from Woodburn's White Dent, is grown in southern Ohio and Indiana.

United States selection No. 193, which is an 8-rowed yellow flint, selected and developed by the United States Department of Agriculture from Hall Gold Nugget, is grown in the Hudson River Valley in southeastern New York. The corn samples were ground in an electric buhr-mill and passed through a 40-mesh sieve.

DESCRIPTION OF METHODS

(1) The total nitrogen content was estimated according to Kjeldahl's method.

(2) The protein nitrogen was estimated according to Stutzer's method (*17*).

(3) The nitrogen of amino acids was determined according to Sörensen's method (*16*), as applied by the writer elsewhere (*5*, *6*, *7*).

Other methods and their details will subsequently be described in this paper.

From the experimental data presented in Table I it will be seen that whereas the Four County corn is characterized by the highest percentage of total and of protein nitrogen, of dry substance and of ash, Hall Gold Nugget is characterized by the lowest percentage of the corresponding constituents; the figures for United States selection No. 77 are between these two, with the exception of the dry substance, which is lower in United States selection No. 77 variety than in Hall Gold Nugget.

TABLE I.—*Percentage of dry substance, of ash, and of total, protein, and nonprotein nitrogen in the ungerminated maize kernel*

Variety of corn	Where and when grown	Dry substance	Ash	Total nitrogen	Protein nitrogen		Nonprotein nitrogen	
		Air-dry corn	Oven-dried corn	Oven-dried corn	Oven-dried corn	Total nitrogen	Oven-dried corn	Total nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Four County corn	At Iowa Agricultural Experiment Station, Ames, Iowa, in 1921.	91.34	1.40	1.74	1.62	95.22	0.081	4.78
	do	91.51	1.39	1.67	1.62	95.31	0.080	4.69
	do	91.41	1.40	1.66	1.64	96.58	0.058	3.42
	do	91.51	1.40	1.71				
Average	do	91.44	1.40	1.70	1.63	95.70	0.073	4.30
United States selection No. 77.	At Piketon, Ohio, in 1919.	90.05	1.32	1.59	1.51	94.96	0.080	5.04
	do	89.95	1.32	1.58	1.52	95.85	0.066	4.15
	do	90.52	1.30	1.59	1.52	95.85	0.066	4.15
	do	89.50	1.33	1.59	1.53	96.14	0.061	3.86
	do		1.34	1.60				
	do		1.33	1.60				
	do		1.32	1.55				
	do			1.61				
Average	do	90.00	1.33	1.59	1.52	95.69	0.068	4.31
Hall Gold Nugget selection No. 193.	At Rhinebeck, N. Y., in 1921.	91.40	1.34	1.46	1.40	96.19	0.056	3.81
	do	91.37	1.33	1.45	1.39	95.26	0.069	4.74
	do	91.37	1.33	1.49	1.39	95.46	0.066	4.54
	do	91.38	1.31	1.46	1.39	95.46	0.066	4.54
	do			1.47				
	do			1.49				
	do			1.42				
	do			1.45				
Average	do	91.38	1.33	1.46	1.39	95.59	0.064	4.41

Experiments conducted with the three corn varieties in order to ascertain how best to extract the nitrogen of the corn flour have shown that the nitrogen extracted by water at room temperature in one hour is as great as that obtained in two and four hours, respectively. In this connection it was found, as would be expected, that a cold aqueous extract of the flour is absolutely free from starch and is very convenient to handle, while a hot aqueous extract contains great quantities of starch and is considerably harder to handle. Since the data concerning the extraction of the flour with water are quite uniform, they are omitted here to save space. It may suffice to mention that in the experiments reported subsequently definite amounts of flour with measured quantities of water were agitated by means of a shaking machine for one hour at room temperature, an antiseptic being used to prevent bacterial action.

The extract obtained was freed from starch, proteins and other insoluble substances by filtration, the filtrate concentrated *in vacuo*, any precipitates formed during concentration were removed by centrifugation, the supernatant liquid evaporated to dryness, and the residue extracted with 80 per cent alcohol, filtered and the alcohol distilled off. The residual yellow sirup was dissolved in hot water, heated to boiling, acidified with acetic acid, boiled, and filtered, the filtrate treated with freshly made lead hydroxide and some lead acetate, and again boiled and filtered. This filtrate was now concentrated under reduced pressure to a small volume.

It should be borne in mind, however, that a water-extract of seeds, prepared in the cold, usually contains proteolytic enzymes which may in part hydrolyze the proteins and proteoses present in the maize kernel. For this reason it seemed necessary to carry out parallel experiments under conditions which destroy the enzymes completely, in order definitely to decide whether or not the amino acids and polypeptides are preformed in the maize kernel. Hence weighed quantities of flour were treated in flasks with boiling hot ammonia-free water and kept on the water bath for about half an hour, whereupon their contents were contrifuged. The solid residue was then treated in the same way once more. The hot water-extracts thus obtained were then treated essentially as described above. The concentrated purified extracts obtained were applied to the estimation of the acid amides, amino acids, and polypeptides.

In order to determine the nitrogen of acid amides, the purified extract corresponding to a definite quantity of flour was made up to 100 c. c., of which two portions of 20 c. c., each were oxidized according to Kjeldahl's method to ascertain the amount of nitrogen present. To 50 c. c. of the remaining solution hydrochloric acid was added to a concentration of 20 per cent and boiled for 30 minutes using a reflux condenser. The hydrolysate was evaporated on the water bath to dryness, the residue transferred quantitatively to a Kjeldahl flask and distilled with magnesium oxide, the ammonia thus obtained being titrated with standard acid.

For the estimation of the nitrogen of amino acids and of polypeptides a sufficient quantity of the purified extract, at least twice the amount used for the acid amide determination, was made up to 200 c. c., in two 10 c. c. portions of which the nitrogen was estimated according to the Kjeldahl method. The remaining 180 c. c. were made up to 200 c. c. and divided into 100 c. c. portions *a* and *b*. In portion *a*, freed from carbon dioxide, phosphoric acid, and coloring matter, the amino nitrogen was determined by the formol-titration method (*16*), while portion *b* was employed for the estimation of the peptide nitrogen. Enough hydrochloric acid was added to portion *b* to make a 20 per cent solution and boiled under a reflux condenser for 12 hours, in accordance with the observations of Fischer (*4, p. 53*). The hydrolyzed material was then evaporated on the water bath to dryness, transferred to a Kjeldahl flask, to which magnesium oxide was added and the ammonia expelled by distillation. The residue was then thoroughly extracted with boiling hot ammonia-free water and the filtered extract concentrated to 100 c. c. of which two portions of 20 c. c. each were oxidized according to the Kjeldahl method, while 50 c. c. of the remaining liquid were used for formol-titration. The result obtained here by titration with formaldehyde diminished by the amino nitrogen secured prior *to* the hydrolysis yields the nitrogen of the polypeptides. The data are recorded in Table II.

Examination of Table II shows that the highest proportion of the nitrogen of acid amides and polypeptides is found in Four County corn, while the highest proportion of amino nitrogen is contained in Hall Gold Nugget selection 193, when the latter is referred to the oven-dried kernel and to its total nitrogen. The figures for United States selection 77 fluctuate, some higher and some lower than for

TABLE II.—*Partition of the nonprotein nitrogen in the ungerminated maize kernel*

Variety of corn	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen	Remarks
Data expressed in percentage of the oven-dried maize kernel:	*Per cent*	*Per cent*	*Per cent*	
Four County corn	0.034	0.044	0.070	Hot-water extract.
United States selection No. 77	.018	.042	.052	Cold-water extract.
Hall Gold Nugget selection No. 193	.021	.052	.035	Hot-water extract.
Do	.022	.050	.039	Cold-water extract.
Data expressed in percentage of the total nitrogen of the maize kernel:				
Four County corn	2.00	2.59	4.12	Hot-water extract.
United States selection No. 77	1.13	2.64	3.27	Cold-water extract.
Hall Gold Nugget selection No. 193	1.44	3.56	2.40	Hot-water extract.
Do	1.51	3.43	2.67	Cold-water extract.
Data expressed in percentage of the water-soluble nitrogen of the maize kernel:				
Four County corn	16.58	21.68	34.36	Hot-water extract.
United States selection No. 77	11.64	28.11	34.11	Cold-water extract.
Hall Gold Nugget selection No. 193	11.15	27.57	18.55	Hot-water extract.
Do	11.32	26.51	20.66	Cold-water extract.

the last named variety. The outstanding feature of Table II, in which each figure ordinarily represents the average of at least four individual estimations, is the fact that each of the three varieties contains acid amides, amino acids, and polypeptides in not inconsiderable quantities. From the circumstance that the results obtained with the hot-water extracts do not essentially differ from the data secured with the cold-water extracts it follows that under the conditions of the work, extraction with cold water for but one hour and rapid evaporation of the extracts *in vacuo*, no noticeable hydrolysis of the proteins under the influence of enzymes took place. It further follows that amino acids and polypeptides are preformed in the corn kernel.

In the experiments described, the purification of the aqueous extracts was effected by means of acetic acid, lead hydroxide, and lead acetate. This treatment removes completely the proteins but does not remove the proteose quantitatively. Since the latter is present in the water extracts of corn, though in very small quantity, the idea suggested itself that the hydrolysis of the polypeptides might be accompanied by the hydrolysis of the proteose, in which case the results reported for the polypeptides might be somewhat too high (see the fourth column of Table II). Hence, it seemed necessary to remove quantitatively the proteose along with the proteins before hydrolysis takes place. This was accomplished by the use of phosphotungstic acid. The dry residue of the alcoholic extract of a known quantity of flour was dissolved in water and made up to a definite volume, usually to 100 c. c. or its multiple. After determining the nitrogen by the Kjeldahl method in a small portion, the bulk of the solution was treated with 5 gm. of sulphuric acid mixed with 30 c. c. of a solution containing 20 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. In each case it was ascertained that a slight excess of the precipitant was used. The precipitates formed were filtered, after 24 hours, and washed with a solution made up of 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. The quantity of nitrogen present in the phosphotungstic precipitate was estimated by the Kjeldahl method. The filtrate from phosphotungstic acid precipitate was treated with calcium hydroxide to slight acidity, then with barium hydroxide to slight alkalinity, whereupon it was saturated with carbon dioxide, the whole heated to boiling, filtered, and thoroughly washed with hot ammonia-free water. The filtrate and washings from the phosphotungstate, sulphate, and carbonate of calcium and barium were evaporated in a vacuum, made up to a definite volume, and the amino nitrogen estimated by formol titration, while the peptide nitrogen was determined, on hydrolysis, as already outlined. The results secured are summarized in Table III.

A glance at Table III shows that the prevalent quantity of nitrogen is present in the phsophotungstic acid precipitate which contains the proteins, proteose, and any basic compounds occurring in maize, while the nitrogen proportions of the polypeptides, amino acids, and acid amides follow in the order named, with the exception of Hall Gold Nugget selection 193, in which the proportion of amino acids is

TABLE III.—*Partition of the nonprotein nitrogen in the ungerminated maize kernel (hot-water extraction and phosphotungstic-acid method)*

Variety of corn	Nitrogen in phosphotungstic precipitate	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Data expressed in percentage of the oven-dried maize kernel:	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Four County corn	0.076	0.032	0.045	0.069
United States selection No. 77	.061	.019	.040	.050
Hall Gold Nugget, selection No. 193	.076	.021	.051	.036
Data expressed in percentage of the total nitrogen of the maize kernel:				
Four County corn	4.47	1.88	2.65	4.06
United States selection No. 77	3.84	1.19	2.52	3.14
Hall Gold Nugget selection No. 193	5.21	1.44	3.49	2.47
Data expressed in percentage of the water-soluble nitrogen of the maize kernel:				
Four County corn	37.35	15.61	21.97	34.07
United States selection No. 77	40.11	12.18	26.67	32.79
Hall Gold Nugget selection No. 193	39.57	11.20	26.95	19.17

higher than that of the polypeptides. From the fact that the percentage of acid amides, amino acids, and polypeptides obtained by the phosphotungstic acid method is not essentially different from the results secured by the first method (without phosphotungstic acid treatment), it follows that under the conditions outlined the amount of proteose in the aqueous extract of the maize kernel is quite insignificant.

It seemed of certain interest to compare the results at hand with those found for the wheat kernel. The average figures of Table IV were calculated from the Tables II, III, and IV of the paper on wheat (*8*).

TABLE IV.—*Partition of the nonprotein nitrogen in the ungerminated wheat kernel.*

Variety of wheat	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Data expressed in percentage of the oven-dried wheat kernel:	*Per cent*	*Per cent*	*Per cent*
Fultz	0.026	0.032	0.084
Kanred	.053	.067	.111
Kubanka	.052	.041	.155
Marquis	.058	.054	.151
Data expressed in percentage of the total nitrogen of the wheat kernel:			
Fultz	1.46	1.77	4.67
Kanred	1.88	2.35	3.89
Kubanka	1.72	1.35	3.13
Marquis	1.91	1.77	4.98
Data expressed in percentage of the water-soluble nitrogen of the wheat kernel:			
Fultz	8.76	10.66	28.09
Kanred	12.99	16.25	26.86
Kubanka	12.61	9.91	37.76
Marquis	12.33	11.46	32.20

When Tables III and IV are examined it is readily seen that, with but few exceptions, the nitrogen of amino acids is higher in the maize kernel than in the wheat kernel, while the reverse is true of the peptide nitrogen. As to nitrogen of acid amides the figures are rather fluctuating, the total difference between the maize and the wheat kernel being not very considerable.

CONCLUSIONS

Polypeptides and free amino acids, which have been shown in this paper to be present in the ungerminated maize kernel, are performed in it.

The amino nitrogen in the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193, makes up, respectively, 0.045, 0.040, and 0.051 per cent calculated on the basis of the oven-dried kernel, and 2.65, 2.52, and 3.49 per cent calculated on the basis of the total nitrogen.

The peptide nitrogen in the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193 makes up, respectively, 0.069, 0.050, and 0.036 per cent calculated to the oven-dried kernel, and 4.06, 3.14, and 2.47 per cent calculated to its total nitrogen.

The proportions of acid amide nitrogen in the ungerminated kernel of the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193 are, respectively, 0.032, 0.019, and 0.021 per cent calculated on the oven-dried kernel, and 1.88, 1.19, and 1.44 per cent calculated on its total nitrogen.

The varieties Four County corn and Hall Gold Nugget selection 193 are, respectively, characterized by the highest and the lowest percentage of their total nitrogen, as well as of their protein nitrogen.

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